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BULLETIN
OF
THE JOHNS HOPKINS HOSPITAL

(THE PUBLICATION OF THE MEDICAL SCHOOL AND HOSPITAL)

(SUPPORTED BY THE DE LAMAR FUND OF THE JOHNS HOPKINS UNIVERSITY)

Vol. XXXV—No. 395] BALTIMORE, JANUARY, 1924 [Price, 50 Cents

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Entered as Second-Class Matter at the Baltimore, Maryland, Postoffice.
Acceptance for mailing at special rate of postage provided for in Section 1103, Act of October 3, 1917. Authorized on July 3, 1918.

BLOOD VOLUME IN PREGNANCY

By H. J. Stander and A. N. Creadick

(From the Department of Obstetrics and Gynecology, Yale University, School of Medicine)

I. A Method for the Determination of Carbon Monoxide in Weak Dilutions

One of us has recently shown that pregnancy is accompanied by a definite increase in the blood moisture, and by variations in the amount of certain organic compounds per unit volume of maternal blood. In view of these findings it seemed advisable to express any variation in the chemical constituents of the blood in values per unit of body weight; and in order to do this we must have definite information regarding the total blood volume of the patient during pregnancy.

There are several methods for the determination of blood volume. The oldest is that of Welcker, who in 1854 determined the blood volume of animals by bleeding them to death and then extracting all the hemoglobin contained in the tissues. Bischoff continued his investigations of blood volume in criminals in a similar manner. Welcker's is the only direct method, but as it is rarely available, numerous indirect procedures have been suggested, since they can be employed on the living animal.

The indirect methods may be classified under two headings, (a) those in which the blood is either diluted or concentrated and the changes in corpuscles or hemoglobin content noted; and (b) those in which a known amount of a foreign substance is introduced into the blood stream and its concentration determined subsequently.

Cohnstein and Zuntz maintained that when isotonic sodium chloride solutions are introduced into the blood stream they diffuse very slowly into the body tissues, and with this in mind developed their infusion method for the determination of blood volume. For this purpose they count the blood corpuscles before and after the infusion of a known amount of sodium chloride solution, and from the difference estimate the volume of blood. Plesch modified their method by estimating the hemoglobin in-

stead of counting the corpuscles. The concentration methods of Tarchanoff and Quincke do not seem to give such constant results as does the dilution method. The basis of these concentration methods, more recently developed by Lindemann, rests on the addition by transfusion of definite amounts of blood of known hemoglobin content. This method, however, is available only in anemic patients. Underhill and Ringer have demonstrated the occurrence of rapid changes in total solids and hemoglobin during disease, and claim that the utilization of such estimations affords a rapid clinical method for determining relative blood volume changes.

In the second group of indirect methods foreign substances such as vital dyes, antitoxin, acacia and carbon monoxide have been employed. The well-known "vital red" method of Keith, Rowntree and Geraghty, the acacia method described by Meek and Gasser, and the use of antitoxin by von Behring, give information regarding the quantity of plasma only; whereas in the carbon monoxide method the total volume of corpuscles is determined.

Grehant and Quinquaud were the first investigators to use the latter method which has since been employed by Haldane and Smith, Oerum, Douglas, Boycott and Douglas, and Plesch. Particularly since the development of the carbon monoxide technique described by Van Slyke and Salvesen, which involves the gasometric determination of small quantities of CO in the blood by means of the Van Slyke apparatus, this method is readily adapted to clinical use.

The present blood volume methods give only relative values, but these are of importance in the study of certain clinical problems. It is possible, for instance, to study the blood volume changes in an animal or human being during such a physiological process as pregnancy. When certain factors, such as the mixing time, are kept constant, one can obtain fairly accurate results by the use of the CO-method. The blood volumes obtained by this method, together with simultaneous corpuscular counts and hematocrit readings, give a fair index of the total blood volume.

The breathing apparatus which we employ for this purpose is constructed after that of Haldane and Smith, and has a capacity of 800 c.c. Part II of this paper explains its main features. As will be seen, the carbon monoxide intake comes quite close to the mouth of the subject, a modification which seems absolutely essential, both because of the tendency toward dissociation of CO in the presence of a high O_2 content, as well as the desirability of reducing the quantity of CO in the residual air to a minimum. Haggard recommends the use of the mouth-piece of the gas mask for human subjects, but in animals we employ a tight-fitting rubber cone, as shown in the diagram. When placed over the nose and mouth, this cone can be made air-tight by strapping with adhesive plaster.

In order to determine accurately the amount of car monoxide that is taken up by the blood of the subjec is necessary to know how much, if any, of the carbon m oxide introduced remains in the respiratory apparatu the conclusion of breathing. This Van Slyke and Sa sen did by shaking the air in the apparatus with bl and estimating colorimetrically its percentage satura with carbon monoxide. Since the blood from differ species and different individuals shows different diss ation curves for the carbon monoxide hemoglobin, it necessary to determine such a dissociation curve for blood of each individual.

Teague used the iodine pentoxide method for the de mination of carbon monoxide in air contaminated w motor exhaust gas, and showed it to be accurate to wit one part in 10,000. Henderson and Haggard suggest to us the application of this technique and, with certa modifications, we have employed it for determining t amount of carbon monoxide left in the respiratory cha ber. Figure 1 is a diagram of our apparatus used f this purpose.

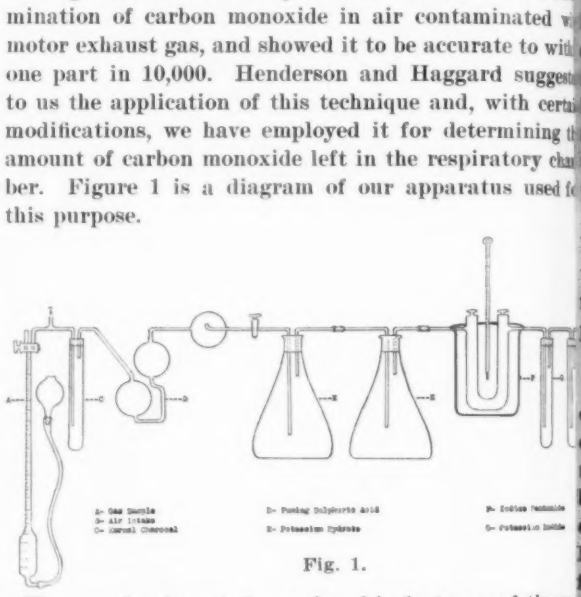

Fig. 1.

The sample of gas to be analyzed is first passed throu kernel charcoal, then through fuming sulphuric acid an followed by a wash with strong potassium hydroxid The gas is then passed through a U-tube containing i dine pentoxide, heated to a temperature of 150° C., an then into an absorbing tube containing a 10% soluti of potassium iodide.

The kernel charcoal is employed for the purpose of a sorbing hydrogen gas, whereas the fuming sulphuric ac takes out methane and the other hydrocarbons. In ord to prevent the fumes of the Nordhausen sulphuric ac from entering the U-tube containing the iodine pento ide, two traps containing strong potassium hydroxi are interposed. When the gas reaches the U-tube, reacts with the pentoxide according to the equation:

$$I_2O_5+5CO=5CO_2+I_2$$

The liberated iodine can then be collected in the pota sium iodide solution and titrated against a standard sol tion of sodium thiosulphate. This solution of sodiu thiosulphate was standardized against an iodine soluti of known strength; so that 1 c.c. of the former was equi alent to 0.03795 of CO at 0° C. and 760 mm. pressure.

In order to procure a very sharp end-point in the titration for iodine, the directions of Low were followed in the preparation of a starch indicator. A cold saturated solution of sodium chloride is made in distilled water and filtered. To 50 c.c. of this solution 10 c.c. of 80 per cent acetic acid and 0.3 grams of starch are added, and the whole mixed cold. The solution is then boiled for two minutes or until nearly clear. A little water is added to replace that lost by boiling, usually about 2.5 c.c. The solution is then cooled and can be used at once. It keeps indefinitely and gives a much sharper end-point than that prepared in the usual manner.

The greatest difficulty in employing this method has been the conditioning of the iodine pentoxide. This substance bought commercially is very unstable and has to be heated for weeks at a very high temperature (230° C.) before it ceases to liberate iodine. Teague, in a private communication, told us that he had encountered the same difficulty and suggested that we use the pentoxide prepared by the chloric acid method described by Lamb and Bray. These authors referred us to the Fixed Nitrogen Laboratory which was kind enough to give us a supply of this material. The carbon monoxide used in these experiments we prepared from oxalic acid by the action of sulphuric acid.

$$C_2O_4H_2+H_2SO_4=(H_2SO_4+H_2O)+CO_2+CO$$

The first of the accompanying tables gives the results of this method for the determination of the percentage of carbon monoxide in our CO-reservoir. Four-hour blanks, run on air introduced into the apparatus from outside the building in order that no carbon monoxide contamination could be possible, showed no trace of liberated iodine. Blanks for longer periods were then run, and the results for the 24-hour blanks are shown in Table II. Provided the rate through the apparatus is constant, the blanks for any given period of time afford absolutely uniform results.

TABLE I.

Sample	Amount of Sample	Amount of CO	Percentage CO
4-hour Blank	—	0	0
CO-Reservoir	2.90 c.c.	1.871	64.5
4-hour Blank	—	0	0
CO-Reservoir	1.12 c.c.	0.722	64.5
4-hour Blank	—	0	0
CO-Reservoir	7.24 c.c.	4.671	64.5

TABLE II.

Blank	Amount of iodine liberated in terms of CO
24 hour	0.277 c.c.
24 hour	0.278 c.c.
24 hour	0.280 c.c.
24 hour	0.278 c.c.
24 hour	0.278 c.c.

TABLE III.

Gas sample	Estimated amount of CO	Amount of CO found
3.35 c.c.	0.337 c.c.	0.338 c.c.
2.03 c.c.	0.283 c.c.	0.284 c.c.
2.65 c.c.	0.369 c.c.	0.368 c.c.

The third table represents our attempt to verify the accuracy of the method. This was done by admitting known quantities of CO into the apparatus for analysis. It should be stated that the results obtained from an Orsat apparatus are not accurate enough when dealing with carbon monoxide in exceedingly small dilutions.

The samples of gas used in the experiments reported in Table III were made up with known dilutions of carbon monoxide, and the total amount of carbon monoxide in each sample estimated. The results demonstrate clearly to what extent the accuracy of this method can be depended upon.

Conclusions

We believe that the above method is sufficiently accurate to determine carbon monoxide in weak dilutions such as are encountered in the estimation of blood volume by the CO-method.

II. Blood Volume Analyses by the CO Method on Normal and Diseased Dogs

Before applying our modifications of the Van Slyke and Salvesen CO-method of blood volume determination to human patients, it was necessary to test its accuracy and safety on experimental animals in physiological and abnormal states. Since our aim was primarily to check the method, no additional cell counts, hematocrit readings nor dye estimations were attempted.

That animals subjected to such experiments should be free from fear, excitement or compulsion has been emphasized by Dale and Laidlaw, Lamson and others. In order to obviate such sources of variation our animals were kept in suitable kennels in the laboratory, where they were attended by the person who was later to conduct the experiment. They were fed regularly in moderate amounts, weighed at the same time in relation to feeding and the onset of the experiment, as well as gradually accustomed to the positions and necessary handling.

Mr. H. W. Antz, technician in this laboratory, built a compact and portable cabinet to contain the respiratory chamber which was constructed on the old Haldane model. This cabinet was 3′×3′×1′ in size, contained all the requirements for one experiment, and was mounted on a wheeled base.

The mouthpiece of the apparatus is an inverted Hygeia nipple, which fits snugly over the nose and mouth of a dog where it is held in place and rendered air-tight by wide adhesive strips. This mouthpiece can be replaced by one

of the type used in the British gas mask when the apparatus is converted for tests on human beings, and this is the only alteration necessary for such a purpose.

The respiratory apparatus consists of a mixing chamber close to the mouthpiece, a Woulff bottle, which contains stick-KOH to absorb the CO_2 expired, and a bladder allowing for the respiratory wave. The whole train is closed at the opposite end by an attachment to an oxygen cylinder by means of which O_2 may be replaced and the contents of the chamber maintained at a uniform tension.

Two gas burettes enter the mixing chamber through 3-way stopcocks. One of these burettes contains CO from the gasometer reservoir and delivers a measured amount of this gas close to the mouthpiece. The other burette is used to withdraw a sample of the residual air from the apparatus at the conclusion of the experiment for determination of the amount of unrespired CO.

Conveniently disposed about the cabinet are a Record syringe and needles for venepuncture, a porcelain dish in which these may be sterilized, iodine, alcohol, and other accessories. On one shelf are several Erlenmayer flasks containing oil, under which samples of blood withdrawn may be protected until the gas analyses are done.

Method.—One gas burette was loaded with a known amount of CO from the gasometer reservoir, the hypodermic needles were sterilized, the air chamber blown out with a draught of pure air and closed when the bladder was distended to a level marked on the scale. This was known to register the air capacity of the apparatus (760 c.c.). Temperature and barometric readings were then taken; the nose-piece was greased with vaseline quickly applied over the nose and mouth of the animal, and fastened in place with adhesive strips; at the same instant the clamp on the tubing was released. No visible alteration of the respiratory rate was noticeable. The capacity of the apparatus was maintained at 760 c.c. by the gradual introduction of a small amount of oxygen; and at a period when the animal was quiet and respiring evenly the carbon monoxide was introduced into the mixing chamber. That the whole amount of CO was inspired was proven by the examination of the residual air in the chamber at the conclusion of the experiment. The air was found to be free from the foreign gas as long as the amount of O_2 introduced was not in excess of 25%. At different periods samples of blood were removed from t femoral vein of the animal. A uniform content of was found in the circulation at the end of ten minu after the gas had entered the chamber; therefore, t blood specimens analyzed were all taken at that inter As soon as the specimen of blood was secured, the ani was removed from the apparatus and the outlets clo by clamps.

TABLE IV.
Normal dogs.

Date	Time	Weight in kgms.	Total cell vol. in c.c.	Cell vol. per 100 gms. body wt.
12/29/20	3:18 P. M.	10.28	487.9	4.74
12/31/20	3:17 P. M.	9.45	387.5	4.10
1/13/21	2:30 P. M.	10.75	472	4.59
1/20/21	2:50 P. M.	11.80	460	3.92
1/ 6/21	4:00 P. M.	6.20	232.5	3.75
1/ 8/21	10:00 A. M.	10.40	473.2	4.55

The accompanying table (IV) shows the results of d ferent readings on animals in a uniform state of heal These animals gave cell volume readings of from 3.75 c per 100 grams of body weight to 4.74 c.c. per 100 grams body weight (average 4.24). While these figures are e tremely uniform, it is of interest to note that the low reading was found in the thinnest dog. All the dogs we in a good state of health, but this animal, although abo the same size as the others, was less well nourishe About the time our protocols were completed, but befo the tables had been prepared, the articles of Whipp Arnold, Carrier and Smith appeared, and it is gratifyi to learn that their readings for normal animals fall wit in the limits of our results (4.20).

At about this time we received an animal in a ver emaciated condition and gravely ill. The animal h "scours" and was maintained alive in isolation for thr weeks, at the conclusion of which time it was necessa to kill her in order to prevent the dissemination of t disease throughout the kennels. Analyses of the blo of this animal gave astonishing results (Table V.). T readings showed 8.6 and 8.9 c.c. of cell volume per 1 grams of body weight.

TABLE V.
Abnormal dogs.

Date	Time	Weight in gms.	Total cell vol. in c.c.	Cell vol. p 100 gm body w
12/30/20	3:45 P. M.	7260	653.0	8.9
1/18/21	3:30 P. M.	7380	631.2	8.6

Discussion

Relative blood volume changes are probably rap (Mendel and Smith) and of clinical significance (Und hill and Ringer). We know little concerning the activ tion of dilution and concentration of the current. R cells probably find a storehouse in the liver (Lamson and white cells in the peripheral arterioles and capilla ies. Undoubtedly there is a difference between t plasma to cell ratio in the content of the larger blo channels, as compared with that of the peripheral ve sels. This last fact would seem to explain the variatio in blood volume figures attained by the several dire and indirect methods. As Whipple and his collaborato suggest, the dye methods measure the plasma, the c method (CO) measures body hemoglobin, and the tot blood volume must be the sum of the two. It is fallacio to assume that the ratio of cells to plasma is constan

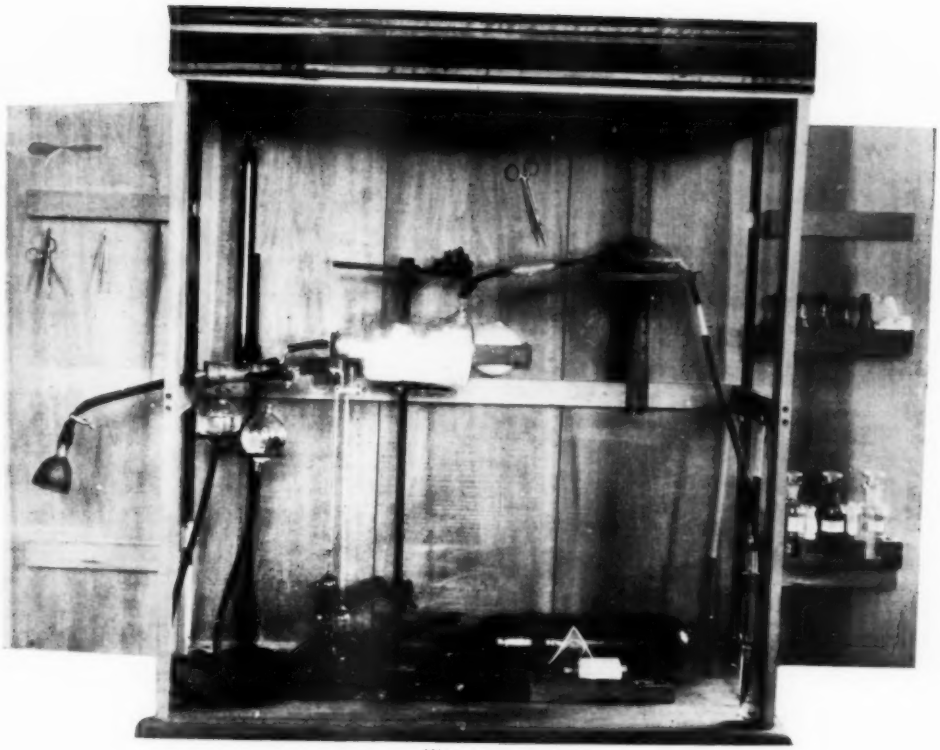

Fig. 2.

The animal studied in Table V. of our series had suffered extreme emaciation and severe loss of body fluids by reason of diarrhea, and the blood volume readings we obtained—8.6 c.c. per 100 gms. body weight—gave twice the normal ratio and might be explained by either the loss of weight or by a marked concentration in circulating fluid. The existence of the two variables convinced us that the CO method was not of itself adequate as a measure of blood volume unless coupled with a concentration test such as a dye injection, hematocrit or hemoglobin estimation. On the other hand, simple concentration tests, such as hematocrit or hemoglobin estimations, may show relative changes in blood volume, but give no absolute estimate of total volume, nor are they useful in comparison in series of individuals.

Bleeding and transfusion tests for total volume are associated with many subsidiary factors which as yet we cannot control. It therefore seems logical to claim that the most serviceable method of estimating total blood volume will prove to be simultaneous readings of a cell method and a plasma method. For the former we have devised a portable apparatus and a refined CO estimation practicable for the bedside. For the latter the dye method of Keith, Rowntree and Geraghty is perhaps the most suitable of those thus far devised.

III. Application of the CO Method to a Pregnant Animal

Before the unforseen interruption of our work we succeeded in completing CO cell volume readings throughout pregnancy in one animal. Obviously, more complete data should have been secured on this and similar animals for any determinate conclusions. However, the readings are of interest and, so far as they go, corroborate Stander and Tyler's moisture and ash determinations.

TABLE VI.

Pregnancy.

Date	Time	Weight in kgms.	Total cell vol. in c.c.	Cell vol. per 100 gms. body wt.
12/29/20	3:18 P. M.	10.28	487.9	4.7
1/13/21	2:30 P. M.	10.75	472	4.4
3/12/21	Lined to male.			
4/12/21	11:50 A. M.	13.80	452	3.28
4/14/21	9:30 A. M.	13.80	443	3.21
4/15/21	Delivered of 5 puppies whose weight aggregated 2500 gms.			

Three interesting items are apparent, (a) the gain in weight, (b) the reduction in total cell volume, and (c) the very low cell volume reading per 100 gms. body weight. Of course there has accrued the usual gain in weight associated with pregnancy. This is made up of the fetal element (2.5 kilos), the membranes and amniotic fluid, and uterine hyperplasia, all in addition to the usual general increase in adiposity associated with normal gestation.

The decrease in cell volume per 100 gms. of body weight might therefore be explained by deducting the weight of the puppies *in utero;* 13.8—2.5=11.3 kilos=4 c.c. cell volume, which is within the limits of normal. However, it is less easy to explain the reduction in total cell volume on such grounds, particularly as carbon monoxide may diffuse through the placental partition and include a measure of the total volume of both maternal and fetal circulations.

Again, it seems likely that the explanation lies in an increase in fluid content of the circulating medium which can be proven only by simultaneous plasma and cell volume readings on a long series of animals. It is our aim to resume the application of these methods, particularly with the enlightenment of Whipple's work, to adult women throughout normal and abnormal pregnancies. Hemoglobin estimations, hematocrit readings, dye or hemoglobin injections for plasma readings should be done simultaneously with carbon monoxide estimations of cell volume. Our method for the last mentioned determination may be applied to human beings with safety and accuracy.

It will also be necessary to determine the blood volume curve following delivery, in order to decide in what fashion it reaches its normal level.

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REPORT OF A CASE OF MALTA FEVER ORIGINATING IN BALTIMORE, MARYLAND

By Chester S. Keefer, M. D.

(From the Biological Division of the Medical Clinic of the Johns Hopkins University and Hospital)

Introduction

During the fall of 1922, a case of Malta Fever came under observation in the medical service of the Johns Hopkins Hospital. The patient was a resident of Baltimore, Maryland, and had never been out of the State of Maryland nor even out of the city for several months. The clinical course of the disease was typical of Malta Fever and the specific organism was isolated from the circulating blood upon numerous occasions and over a considerable length of time. The infrequency of this infection in the United States and particularly in the East, the difficulties in the diagnosis of sporadic cases and the importance of recognizing such cases, seem to warrant this report.

Synonyms and Etiology

Malta fever has been recognized for years and has been known in the literature under a variety of different names, such as: Undulant fever or Febris undulans (from the wave-like accessions of fever); Malta, Maltese, Mediterranean, Gibraltar or "rock," Neapolitan and Cyprus fever (from the geographical distribution); Slow, Mountain, Continued, Goat fever (in Texas and New Mexico); Mediterranean phthisis (from the bronchitis, anemia and night sweats suggestive of phthisis) Bruce's septicæmia, Melitensis septicæmia, Febris melitensis, Mittelmeerfieber, etc.

The discovery of the causative agent was made by Bruce [1] in 1886. At that time he isolated an organism, which he called *Micrococcus melitensis,* at autopsies of several fatal cases. In 1887,[2] he further proved his observations and since then the etiology of this disease has been certain.

In the most recent literature the organism has been known as a bacillus and not as a micrococcus. The development of the term bacillus was as follows: In 1898, Durham [3] noted that a bacillary form of *melitensis* occurs and believed that conditions of medium and temperature determined whether or not the forms would be bacillary or coccoid.

Eyre,[4] on the other hand, considered the rod-like organisms occurring in cultures as involution forms.

Until 1918, the organism was termed in most articles *Micrococcus melitensis,* although in some textbooks it was designated as a bacillus. At this time (1918) Evans[5] once more called attention to the fact that in young cultures the organism of Malta fever was a rod form and suggested, at that time, that the organism be called *Bacterium melitense.* Her results were confirmed by Meyer and Shaw [6] and by other workers in this field.

The explanation for the generic name *Micrococcus* which Bruce gave to his organism is based upon the interesting work of Evans.[7] By comparing a number of strains of *melitensis* she showed that there are some strains which appear entirely as coccoid, while others appear entirely as bacillary, the latter being by far the more common. Hence, it would appear probable, as Evans suggests, that Bruce was working with strains which were coccoid in form and which were of peculiar morphology as judged by the majority of the strains in this group. In spite of the difference in the morphology there is no question but that the organisms belong to the same species, because both the coccoid and bacillary forms are identical in cultural and biochemical reactions and cannot be differentiated by simple agglutination tests.

A further discussion of nomenclature and classification will be given later.

The following case was observed by us over a period of six weeks:

CASE REPORT

A. S.—(Med. No. 48513); Aged 19; Male; White; Laboratory technician. Admitted, October 18, 1922; Discharged, December 10, 1922.

Complaint.—Evening fever and chills for three weeks.

F. H.—Unessential.

P. H.—The patient has always enjoyed excellent health until his present indisposition. He has never had any serious illness. No history of typhoid fever, pneumonia, malaria or rheumatic fever. One year prior to his present illness he had an acute maxillary sinusitis which was promptly and completely relieved by proper treatment.

His habits have been exemplary. He has not been out of Baltimore City for several months, and then he was not out of the state but attended a National Guard Encampment. He has never drunk goat's milk. He has not been exposed, so far as he knows, to typhoid fever, tuberculosis, or malaria. He has been working as a technician in the histological laboratory of the Medical School but no bacterial cultures have been used in his work, and no goats have been kept in the laboratory.

He has always been very fond of milk and has been in the habit of eating considerable quantities of cheese.

Present Illness.—Four weeks before entering the hospital the patient began to suffer from frontal headaches which came on most frequently in the late afternoon and early evening. The headaches were soon followed by and associated with evening fever and chilly sensations. His headaches became less severe and seemed to be relieved somewhat by wearing glasses which corrected a slight myopia.

Three days before admission to the clinic, he developed pain and distress in the epigastrium which was accompanied by nausea but no vomiting, and his temperature, taken that evening, was 102° F. With these abnormal sensations he had chilliness, moderate prostration, restlessness, insomnia and headache; upon several occasions he had definite chills and drenching sweats. These symptoms were always worse at night but during the day he felt tired and had aching in his joints, particularly, the hip and knee-joints. He did not give up his work, in spite of his indisposition, until he entered the ward.

P. E.—The temperature was 99.2° F. (38° C.). Pulse 72. Respirations, 20 per minute. Blood pressure 120/65. There was evidence of recent loss of weight, but the skin and mucous membranes were of fairly good color and no abnormal eruptions were present. The bones and joints showed nothing abnormal. The lymph glands were not enlarged. Examination of the head and neck revealed nothing abnormal except several carious teeth. The tonsils were not enlarged.

The heart and lungs were clear. The abdomen was soft and the spleen was not felt. Examination of the genitalia, reflexes and nervous system, showed nothing striking. A rectal examination was negative.

Laboratory Examinations.

Blood: R. B. C.....4,348,000
Hb. .....68% (Sahli)—(normal reading 85%)
C. I. 0.8%
W. B. C. 12,000

Differential formula—300 cells

P. M. N.	55 %	6,060
P. M. B.	0.3%	40
P. M. E.	0.7%	80
S. L. & L. L.	34 %	4,620
L. M. & Trans.	10 %	1,200
	100%	12,000

Stained smear showed slight pallor of all the red blood cells. No abnormalities were seen except the increase in lymphocytes and the slight increase in the large mononuclear-transitional group. No malarial parasites were seen in fresh or in stained preparations.

Blood Wasserman.—Negative.

Blood Culture.—Taken the day patient entered was sterile after four days' incubation.

Urine.—Clear; Sp. G. 1,020; no albumin, casts, bile or blood. Test for Urobilin was positive.

X-ray of Chest.—Lungs clear, heart and aorta not enlarged.

X-ray of Sinuses.—Clear.

Widal.—Negative.

Urine Culture.—Negative.

Stool Culture.—Normal intestinal flora.

A very careful search for foci of infection including sinuses, teeth, gastro-intestinal and genito-urinary tract revealed nothing abnormal except several carious teeth.

Summary.—A young man who had been perfectly well until three weeks before his admission to the hospital. The onset of his sickness was gradual, with evening fever, chills, headache and malaise, and was followed by loss of flesh, insomnia, arthralgias and weakness. Upon admission to the hospital the only significant features upon physical examination were slight evidence of loss of weight, slight pallor, several carious teeth and slight fever.

Laboratory examinations revealed nothing of great importance at first, with the exception of the blood picture which showed a moderate secondary anemia with a slight leucocytosis and an increase in the lymphocytic, large mononuclear, and transitional elements.

The diagnosis of Malta fever became evident on recovery of the organism from the second blood culture.

Blood Picture.—The blood counts with the differential formulæ are given below. The striking features are a slight grade of secondary anemia with normally appearing red blood cells and a white blood cell count which varies from six to twelve thousand with a relative and absolute increase in the lymphocytes as high as 52 per cent with an increase in the large mononuclear-transitional group as high as 22 per cent.

Castellani and Chalmers state that the lymphocytes may be increased to 80 per cent in some cases, but that all cases show an increase in the percentage of the lymphocytes, both relative and absolute. Hayat,[8] Schoull,[9] Gentry and Ferenbaugh[10] and Bassett-Smith[11] have

Blood picture.

Date	R. B. C.	Hb.	C. I.	W. B. C.	P. M. N.	P. M. B.	P. M. E.	S. L. & L. L.	L. M. & Trans.
10/18/22	4,348,000	68%	0.8	12,500	55%	.3%	.7%	34%	10%
10/21/22	4,000,000	70%	0.8	6,540	31%	0	1%	57%	10%
10/23/22	4,000,000	68%	0.8	7,100	46%	0	0	40%	13%
10/26/22	4,276,000	70%	0.8	9,760	49%	0	0	43%	8%
10/30/22	4,000,000	75%	0.9	8,440	53%	0	0	38%	7%
11/ 3/22	4,272,000	75%	0.9	8,040	54%	0	0	43%	3%
11/ 8/22	4,072,000	90%	1	7,200	54%	0	0	43%	3%
11/15/22	4,024,000	65%	.8	7,760	55%			34%	11%
11/21/22	4,080,000	70%	.8	8,800	41%	1	0	43%	15%
11/29/22	3,684,000	65%	.9	8,760	43%	0	0	48%	9%
12/ 1/22	4,192,000	75%	.9	6,080	26%	0	0	52%	22%
12 /6/22	4,024,000	68%	.8	6,800	46%	0	1	46%	8%
12/10/22*	4,496,000	80%	.9	9,360	35%	1	3	50%	11%
4/25/23†	5,100,000	95%	.9	6,400	72%	1%	1%	23%	3%

* Afebrile, but blood cultures are still positive.
† Complete recovery.

studied the blood of many patients quite carefully and conclude that there is a secondary anemia with a loss of 20 to 40 per cent of corpuscles, with a slight poikilocytosis and a reduction of hemoglobin in greater proportion than the red cells. They state that the leucocyte count is normal but a decrease in the polymorphonuclear neutrophiles and an increase of mononuclears are always present.

In the cases reported by Craig [12] there was a moderate leucocytosis with a slight increase in the P. M. N. However, the majority of observations confirm the former findings.

Urine.—The urine was of normal specific gravity. There was slight albuminuria during the marked febrile period with occasional hyaline and granular casts and at times rather marked urobilinuria. The albumin, casts and urobilin disappeared as the fever subsided.

Upon one occasion M. melitensis was isolated from the urine. This was at the time when the organisms were most abundant in the blood stream. However, they soon disappeared and were not present upon the patient's discharge from the hospital.

The organism may be recovered from the urine in about fifty per cent of the cases and over a long period of time, usually between the fifteenth and two hundred and forty-ninth day of the disease (Horrocks, Bassett-Smith and Shaw) [13] often in large numbers—*e.g.* 1000 per c.c. Patients often discharge organisms in their urine for a long time after the systemic evidence of infection has disappeared.

Stools.—The stools were normal. The organism was never isolated from the stool, although numerous cultures were made. It is not, as a rule, found in the fæces. Eyre [14] was able to isolate the organism from the colon of a fatal case, but it has never been demonstrated *intra vitam.*

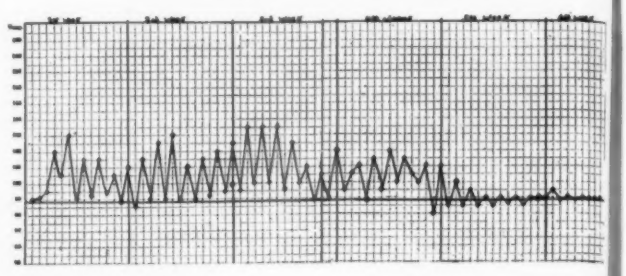
Course in the Hospital

Symptomatic.—The patient's symptoms at all times during his stay in the hospital were mild and consisted chiefly of headaches, attacks of restlessness and irritability. These were most prominent during the height of the fever. He had numerous drenching night-sweats and occasionally definite chilly sensations. He had definite arthralgias, but never any swelling or tenderness of the joints. *He was entirely free from neuralgic pains* and never had any signs of any of the complications of this disease, such as, orchitis, parotitis or neuritis.

He had moderate anorexia and constipation and lost several pounds in weight, felt weak and worn out. Outside of these rather generalized symptoms indicative of a low grade infection, he was not very much disturbed.

Fever.—The fever is best described by the temperature chart below. However, it will be seen from this record of the daily high and low temperatures, taken over a

Temperature Chart.



period of six weeks, that the fever was remittent in character with several waves of rise and fall. One of the remarkable things is that the temperature became normal and the patient improved markedly long before his blood stream was free from organisms.

Recovery was complete. The patient, when seen in September, 1923, had gained thirty pounds in weight and has never had a recurrence of fever or symptoms.

Clinical Description

It is desirable, for purposes that will be brought out later in this paper, to give a short résumé here of the clinical picture of Malta fever and to point out that the course of the disease in our patient was characteristic of the infection as it has been commonly observed in regions where it is endemic.

This is particularly important, because it will be shown that the organism recovered from this patient probably belongs to a strain of Br. melitensis which hitherto has not been known to infect man.

Malta fever varies widely in its clinical manifestations and presents a variety of clinical pictures. There are all variations from the mild ambulatory form to the acute overwhelming septicæmic variety with rapid exitus. But on the whole, most cases have a rather insidious onset and run a protracted course, averaging from three to four months. They are characterized by fever, which may be intermittent, remittent or continuous in type—and there are invariably several relapses. The features indicative of a generalized septic infection are generally present—headache, prostration, asthenia, anorexia, insomnia, sweats and loss of weight. Besides these features, the joint and neuralgic pains are quite characteristic. The joint pains may be quite severe and are increased by motion and pressure; occasionally there is swelling and redness. The swelling may come on quickly, the acute symptoms disappear in a few hours and the joint become entirely well in three or four days. The joints most frequently involved are the hips, knees, ankles and shoulders. The costo-sternal and costo-chondral articulations are sometimes affected and may suppurate, the M. melitensis being isolated in pure cultures (Gilmour [15] Kennedy [16]). As a rule, however, the joints do not suppurate.

A very interesting group of cases has been described by Curry [17] and Craig,[18] in which the outstanding features of the melitensis infection were severe recurring attacks of polyarthritis, with slight fever and anemia. The joint symptoms were so prominent that the cases were diagnosed "subacute rheumatism" and the true nature of the infection was not recognized until specific agglutination tests with the melitensis organism were made.

Another very prominent feature in quite a few cases is the occurrence of very distressing neuralgic pains. These most frequently affect the sciatic nerve, but other peripheral nerves may also become implicated. The nerve pains are somewhat more frequent than the joint pains.

Very rarely in a severe case a liver abscess or a subphrenic abscess supervenes in a case of Malta fever,[19] but as a rule the lesions produced by the organism are non-suppurative in character.

The objective findings generally yield very little besides fever, slight tachycardia, a moderately enlarged spleen and moderate anemia; occasionally an orchitis, arthritis or evidences of a peripheral neuritis may be found.

In summary, it may be said that Malta fever is a disease with a septicæmia and with no very definite localizing lesions. Metastases of a suppurative character are quite unusual. The clinical diagnosis is very difficult and uncertain unless the specific organism can be isolated from the blood, urine or spleen, or unless the blood serum contains specific agglutinins. The prognosis is generally good; the mortality averages from 2 to 8 per cent (Eyre [20]). Death, when it does occur, is due to an overwhelming septicæmia, or to a hyperpyrexia and collapse.

The fatal cases show enlargement and softening of the spleen and lymph nodes with lymphoid hyperplasia, congestion of intestinal mucosa but no swelling or ulceration of Peyer's patches. The specific organism can be isolated from the spleen, lymph glands (mesenteric), gall-bladder, kidneys and liver.

BACTERIOLOGY OF THE PRESENT CASE

Blood Cultures.—The results of blood cultures are recorded below. The blood was cultured in plain broth, in plain agar and in glucose agar. Four plates and one flask of broth were inoculated each time a culture was made and they were incubated at 37.5° C. for from seven to ten days before they were discarded. The technique consisted of withdrawing 20 c.c. of blood in a sterile syringe from an arm vein, inoculating 100 c.c. of broth with 12 c.c. of blood, inoculating each of two tubes of plain agar and glucose agar with 2 c.c. of blood, and pouring plates.

The results were as follows:

Date	Plates Colonies per c.c.	Broth
10/19/22	No growth	No growth*
10/25/22	6 cols.	Heavy growth †
10/27/22	5 cols.	" "
10/29/22	5 cols.	" "
11/ 3/22	4 cols.	" "
11/ 6/22	4 cols.	" "
11/10/22	3 cols.	" "
11/18/22	2 cols.	" "
11/25/22	1 col.	" "
11/30/22	1 col.	" "
12/ 2/22	1 col.	" "
12/15/22	1 col.	" "
12/28/22	1 col.	" "
1/10/23	1 col.	" "
2/10/23	None	No growth
4/25/23	None	" "

* Cultures discarded after 4 days' incubation.
† 7 days' incubation.

The organism isolated from the blood first appeared upon the plates after five or six days' incubation. They were small, non-hemolytic, grayish colonies and upon staining they were found to be Gram-negative bacilli. They were non-motile and did not form spores. They appeared both singly and in short chains of two or more, but long chains were not observed.

The organisms in young cultures were bacilli, but after they had been sub-cultured several times, many of them assumed the forms which suggested cocci rather than bacilli.

The organism grew well after three or four days upon ordinary media such as plain agar, blood agar, plain broth and milk. It changed the reaction of litmus milk to alkaline without coagulation after six days. No gas was formed and no acid produced when the organism was grown in maltose, saccharose, raffinose, levulose, mannite and dulcite. Dextrose and lactose showed an alkaline reaction, but no gas after ten days' incubation.

Morphologically, this organism corresponded to the *Brucella melitensis* group and this was confirmed by specific agglutination reactions.

It will be seen from the foregoing table that the organism was obtained from the circulating blood over a period of at least sixty-seven days and it probably would have been obtained earlier had the first blood culture been allowed to incubate more than four days before it was discarded. It seems quite important, therefore, that in a case of suspected Malta fever the blood cultures should be allowed to incubate for at least seven days before they are discarded as negative. In this instance the organisms did not begin to appear upon the plates until the sixth day of incubation, and even then the colonies were quite small, and the growth in broth was scant.

As a rule, the organism may be isolated from the blood in the majority of cases of Malta fever, particularly at the height of the infection. Shaw [21] obtained 69 positive cultures in 103 cases, Gilmour,[22] 38 positive cultures in 45 cases, Zammitt,[23] 27 positive in 49 cases and Bassett-Smith [24] 16 positive cultures in 24 cases. It is also known that the organism may be isolated from the blood over long periods of time. Gilmour [25] obtained it from the second to the three-hundredth day of the disease in numbers varying from one colony in 5 c.c. to ten thousand colonies per cubic centimeter of blood.

One of the striking features is that the organism may be isolated from the blood of patients long after the fever and symptoms have subsided, and also from persons who have never known that they have had the infection (ambulatory type). In the present case the organisms were isolated from the blood several weeks after the disappearance of fever and symptoms. Shaw also isolated the organism from the blood of nine out of twenty-two apparently healthy dock-hands at Malta.

Serological Tests.—The microscopic method of agglutination was employed and all tests were heated at 56° C. for one hour. Viable organisms suspended in saline solution were used and carefully controlled. The first tests performed were to determine the titre of a known anti-melitensis serum. Six strains were used.*

The results of the tests are tabulated in Table I.

TABLE I.

	1/20	1/40	1/80	1/160	1/320	1/640	Control Saline + Orgns.
Anti-melitensis serum vs. Strain 100...	+	+	+	+	+	+	0
Anti-melitensis serum vs. Strain 101...	+	+	+	+	+	+	0
Anti-melitensis serum vs. Strain 102...	+	+	+	+	+	+	0
Anti-melitensis serum vs. Strain 103...	+	+	+	+	+	+	0
Anti-melitensis serum vs. Strain P1....	+	+	+	+	+	+	0
Anti-melitensis serum vs. Strain P2....	+	+	+	+	+	+	0
Normal serum vs. each of the six strains.....	0	0	0	0	0	0	0

+ Complete agglutination.

Since it was found that the known serum agglutinated several known strains in dilutions of 1-640 with no agglutination in the controls the following series was run:

The patient's serum agglutinated both the known organism and the organism isolated from his blood, both in high dilutions. In the first instance, agglutination was present in a dilution of 1:5120, and in the second instance in a dilution of 1:20,640. The latter was particularly striking on account of the presence of agglutination in such a high dilution and on account of the absence of agglutination in lower dilutions, (none from 1:10 to 1:160). This marked pro-agglutinoid zone, as it is called, seems quite important, since agglutination may be negative if the dilutions are not made high enough. This pro-agglutinoid zone was not as striking when the patient's organism was agglutinated with known anti-melitensis rabbit's serum, nor was it marked when a known melitensis organism was agglutinated with the patient's serum—although it was present, it was only below the dilution of one in twenty.

Pro-agglutinoid zones are more common in B. melitensis serum than in sera from enteric fever and cholera (Birt and Lamb [26]). The nature of this zone of inhibition is unknown. It was found, however, that when the serum was allowed to stand in the ice-box or at room temperature for one week or more, it would lose its inhibitory zone to a great extent, whereas heating fresh serum at 56° C. for two hours did not affect it. However, if recently isolated organisms were used with fresh serum, the zone of inhibition was more marked than when organisms that had been sub-cultured many times were used. Whatever the mechanism may be, it is important to know that the "zone" sometimes exists in high dilutions.

Since 1897, when Wright, Smith and Semple [27] applied the "serum reaction" of Gruber, Durham and Widal to the diagnosis of Malta fever, this method has been used extensively; and it has been shown that specific agglutinins appear in the blood of those suffering from the disease from the fifth day onward, occasionally earlier (Eyre [51]). However, there are cases in which agglutinins do not appear until after the disease is well established. The agglutinins are usually present in high concentration, giving the serum a titre varying anywhere from 1:100 to 1:500,000, usually 1:100 to 1:5000. The agglutinins persist in the blood a long time and may react in dilutions of 1:50 and 1:100 for from three to seven or even ten years after an attack of fever. In the present case the agglutinins were

* Strains and serum obtained from the United States Public Health Service through the kindness of Colonel Craig of the Army Medical School.

TABLE II.

	1/10	1/20	1/40	1/80	1/160	1/320	1/640	1/1,280	1/2,560	1/5,120	1/10,240	1/20,480	Control Saline + Organisms
Anti-melitensis serum (rabbit) vs. Pt. org.	0	+	+	+	+	+	+	0	0	0	0	0	0
Patient's serum vs. Patient's org.....	0	0	0	0	0	+	+	+	+	+	+	+	0
Patient's serum vs. known B. melitensis..	0	+	+	+	+	+	+	+	+	+	0	0	0
Normal serum vs. Pt. org.....	No agglutination												
Normal serum vs. known B. melitensis...	No agglutination												

present in dilution of 1:20,640 during the disease, and five months later they were still present in dilution of 1:320, with a "pro-agglutinoid zone" present up to 1:40.

The reaction is specific for organisms of the B. melitensis * group of which melitensis is a member. Birt and Lamb [26] showed that sera from healthy people and from patients suffering from diseases other than Malta fever never yield a complete reaction in dilution of 1:20. However, it has been shown by Nègre and Raynaud,[52] Nicolle and Conor [28] and others, that many sera will clump the bacteria of the Brucella group non-specifically, if the serum is not heated to 50° C. for one hour. A reaction is not considered indicative of true infection unless there is complete agglutination in sera of 1:100 dilution when heated at 50° C. for one hour. In the series run in this case, all agglutination tests were placed in the water-bath at 56° C. for one hour, in order to avoid the non-specific reaction. However, a parallel series heated at 37.5° C. gave the same results. In a very small number of sera from normal people and people suffering from other diseases (20 cases) the non-specific reaction was not encountered. However, since it is known to occur in some cases, it is best to carry out the tests at 50° C. Complete agglutination of a suspension of organisms by sera in dilution of 1:100, provided proper controls are run, a reliable strain is used and the serum heated to 50° C. for one hour, would be indicative of true infection.

In general, continuously high agglutinin concentration in the blood is prognostically favorable, whereas a sudden fall in titre is an unfavorable sign from the standpoint of duration and recovery.

Source of Infection

The occurrence of Malta fever in the United States is rather infrequent, if one may judge from the reports in the literature.

The first authentic case originating in the United States is the one reported by Craig,[12] in 1905. The patient was a nurse in a hospital in Washington, D. C., and developed a typical infection with fever and specific agglutinins in her blood. The source of the infection was never determined. It was suggested that she acquired the infection from patients in the hospital suffering from the disease, but since there were no cases in the hospital at that time, it seemed unlikely that this was the source of the infection. Along with this case Craig reported nine cases occurring in soldiers who had recently returned from the Philippines. The cases reported prior to this time, if one excludes the laboratory infection reported by Strong and Musgrave,[29] were all cases occurring in patients who had acquired the disease in other countries, such as Porto Rico, The Philippines and Cuba, and had been sent to United States Army hospitals for treatment (Musser,[30] Curry,[17] Craig [16]). In the same year, 1905, a woman, at Athenea, N. J., drank milk from some of the goats aboard the S. S. Joshua Nicholson and developed Malta fever. No other cases were reported in the United States until 1911, when Ferenbaugh,[31] and Gentry and Ferenbaugh [32] reported an endemic of twelve cases occurring in South West Texas along the Rio Grande, Neuces and Pecos River country. All of the patients suffering from the disease gave a history of drinking unboiled goat's milk, or were actively connected with the goat-raising industry. The very interesting fact was brought out by the investigations of these authors that for twenty-five years prior to 1911 there had existed a disease in South West Texas, known as continued fever, slow fever or "goat fever," and characterized by continued fever, frequent relapses, pains in the joints and a low mortality. It was called "goat fever" because it was thought that the infection was acquired from goats. Its true nature was not recognized until 1911, when Ferenbaugh reported his cases.

In 1912 Yount and Lorney [33,34] reported the occurrence of a few cases of Malta fever in Arizona. All of these cases occurred among goat-herds. In 1913 Wellman, Eustis and Schochet [35] reported a case occurring in Louisiana. The patient was a native of Mexico and had lived in Texas for some time. He had continued remittent fever over a long period of time, and his blood serum agglutinated melitensis organisms in dilutions of 1:60 and 1:80. The organism was not obtained *intra vitam* and cultures made from the spleen after death did not show the specific organism, but the authors felt from the clinical features and the agglutination tests that the diagnosis was certain.

In 1918, Woolsey [36] reported a case in Missouri, and, in 1922, Lake [37] reported an endemic among drinkers of goat's milk in Arizona. Aside from these cases, a rather careful search of the literature has brought to light no other recorded cases arising in the United States. Most of the reported cases have occurred in regions where the disease is endemic and where goat's milk is used extensively as an article of diet. There may be many other cases which go unrecognized on account of the mildness of the infection or because serum tests with melitensis organisms are not applied in cases of fever of unknown origin.

The source and mode of infection in the case reported here are unknown. It was impossible to obtain any history of the patient having ingested goat's milk or products made from goat's milk. It was brought out however, that he used, in his diet, large quantities of raw cow's milk and products made from cow's milk. May not the occasional infection of this kind, arising sporadically in an area where there are no goats, be due to

* The genus to which Melitensis, abortus and para-melitensis belong, first suggested by Meyer and Shaw, and Evans.

drinking cow's milk? This immediately brings up the very interesting question of the relation between the organism, occurring frequently in the milk of cows (B. abortus), which causes infectious abortion in cattle, and the organism which is common in milk of goats (B. melitensis) which causes undulant fever in man.

Shaw [38] observed that the sera of 10 out of 33 cows examined in Malta gave a positive agglutination reaction with melitensis, and he was able to isolate the organism (probably B. abortus) from the milk of two cows. Since then Shroeder and Cotton,[39] Zwick and Krage,[40] Fabyan and Theobald Smith,[41] Fleischner and Meyer,[42] and others, have shown that B. abortus can be isolated from the raw milk of cows in a high percentage of samples. Kennedy [43] also called attention to the fact that the milk and blood serum of five out of thirteen cows examined in England contained agglutinins for B. melitensis. All of these facts remained unconnected, until Evans [44] showed in an illuminating piece of work that B. abortus and B. melitensis could not be distinguished one from the other culturally, morphologically, or serologically. Her work was further extended and confirmed by Meyer and Shaw,[45] Feusier and Meyer,[46] and by Khaled,[47] and practically every worker in this field has reached the same conclusions, so that the accumulated evidence up to this time has culminated in the conclusions of Burnet [48] that melitensis and abortus are distinct serological varieties of one and the same species.

It was pointed out by Feusier and Meyer [46] that the melitensis and abortus organisms fell into four distinct serological groups and could be distinguished only by absorption tests. Evans [49] has recently repeated this work, using more strains, derived from human, bovine, porcine, caprine and equine sources, and concluded that this group of organisms forms one large Genus, which she called *Brucella melitensis*, and that the strains could be differentiated into at least seven serological groups, of which four included only one or two strains each and were relatively unimportant. The majority of bovine and porcine strains fell into one large group, which was designated *variety abortus*. Two strains of human origin were of this variety, one being (No. 455) the strain isolated from the blood stream of the patient reported here. The organism was sent to Dr. Evans for study by Dr. H. L. Amoss. The history of the other human strain in this group (No. 426) is uncertain. It was obtained by Dr. Evans from Dr. K. F. Meyer and originally came from the Royal Army Medical Corps of London. It was thought to have been isolated in Austria but this was uncertain; hence the exact history of this organism (No. 426) was not definite.

Two of the small serological groups were so closely related to this large abortus group that they were considered subvarieties. One of these includes only one strain of bovine origin; the other includes two strains, both of human origin.

Another important group included human, bovine, caprine, porcine and equine strains. It was designated *Melitensis A*.

A third group of three strains, characterized morphologically by a predominance of coccoid cells, assumed importance from the evidence that this was the variety that Bruce was working with when he named his newly discovered organisms *Micrococcus melitensis*. It was designated *Melitensis B*.

Evans also concludes that by simple agglutination tests one cannot differentiate between varieties of abortus and Melitensis A. These two varieties can be differentiated from Melitensis B. by the simple agglutination test only when the titre of the serum is accurately known for several varieties. Other facts which support the view that these organisms belong to the same group are: (1) the same type of infection may be produced in animals by organisms of this group derived from different sources; thus, bovine and human strains produce the same type of infection in guinea-pigs (Evans,[5] Khaled,[47] Meyer, Fleischner and Shaw [50]); monkeys, (Fleischner, Vecki, Shaw and Meyer,[50] Khaled [47]), and finally, Evans [53] has produced abortion in a pregnant heifer by injecting organisms derived from a human case of undulant fever. The only difference in the production of the infections is that larger doses of the abortus organism have to be used in order to accomplish the same result as when the melitensis organism is used. This was true throughout except in the experiments of Khaled, who found that the abortus were more invasive than the melitensis organisms.[47] Moreover, Khaled has been able to protect a monkey from melitensis infection by immunizing the animal first with an intravenous injection of a killed suspension of abortus.

That organisms coming from cow's milk may cause the occasional case of undulant fever is suggested by the occurrence of typical cases of undulant fever, as confirmed by laboratory diagnosis, in places where the ingestion of goat's milk is impossible—Egypt (Khaled [47]), England (Kennedy [43]), United States (Craig, and the present case).

Another fact of importance is that Evans [7] has shown that the human strains isolated from Malta fever patients, who have been known to have been infected by drinking goat's milk, fall into the same serological group as the organisms isolated from goats, and are classified as Br. melitensis A group. The group which is designated as the abortus group contains mostly bovine strains. There are, in this group, a few porcine and two human strains.

The one human strain of this group (No. 455) was isolated from the blood stream of our patient. Since this strain falls into the abortus group, it shows that the

organism is more closely related to the abortus than to the melitensis A group, (the group to which belong most of the caprine and human strains).

This fact is quite important, since this is the first recorded case of Malta fever in which the infecting organism has been shown to fall into the abortus group. It also shows that a disease in man corresponding to Malta fever can be caused by an organism belonging to the abortus group. It seems most likely, therefore, that the origin of this infection was bovine and not caprine, and since the organism occurs in the milk of cows, the infection may have had its origin either from cow's milk or from products made from cow's milk.

TERMINOLOGY

Since the discovery of the close relationship which exists between the melitensis-abortus group of bacterial organisms (Evans[5]), great confusion has arisen in regards to the terminology. The whole subject has been reviewed recently by Evans.[55] She proposes the generic name *Brucella* for this group of organisms, as was first suggested by Meyer and Shaw in 1920.[16] This name has already met with approval from foreign investigators (Khaled,[47] Archibald[54]). She suggests that, in abbreviating *Brucella, Br.* be used instead of *B.*, as preferred by Meyer and others, to avoid confusion with *B.*, the abbreviation for the Genus Bacillus. She further proposes that the whole melitensis-abortus group be known as *Brucella melitensis* in accordance with the custom of observing priority of publication in determining the nomenclature. Upon a basis of serological classification she also proposes that the following serological groups be recognized.

1. *Brucella melitensis-abortus,* the group including most of the bovine, porcine and two human strains.

2. *Brucella melitensis melitensis,* the group including chiefly the strains from human and caprine sources. This group may be further designated tentatively groups A, B, etc.

She feels that Group 1 and Melitensis A of Br. melitensis are very closely related, because they cannot be differentiated by simple agglutination methods.

It seems most logical, therefore, that, when speaking of this group of organisms, the term *Brucella melitensis* be used.

Treatment.—There is no specific treatment for Malta fever. Serums and vaccines have been used by various workers in this field with varying results. Upon the whole, serums and vaccines in the treatment of this disease are very discouraging. Since vaccine therapy has been tried with varying success by others, it was tried in our case, although it was difficult to see what vaccine therapy would accomplish in a man who already had antibodies in high concentration in his blood stream and abundant antigen there to produce them. However, large doses of vaccine were given repeatedly and over a period of several weeks. No improvement could be noted; the course of the disease did not seem to be altered; the organisms in the peripheral blood did not decrease, and the agglutinating power of the blood serum was not raised; so that vaccine therapy did not seem to be of any therapeutic effect in this case. The main treatment consisted in the regulation of the patient's activities by keeping him at rest in bed as long as he was febrile, giving him an abundant, nourishing diet and treating him symptomatically, giving sedatives for discomfort and insomnia.

CONCLUSIONS

1. A case of Malta fever occurring sporadically in Baltimore, Maryland, is reported.

2. The clinical features and laboratory findings in cases of Malta fever are presented and reviewed.

3. The organism isolated from this case has been shown to belong to the abortus group, of Br. melitensis, so that the origin of the infection seems to have been bovine and not caprine. This is the first recorded case of a disease in man corresponding to Malta fever, due to an organism belonging to the abortus group.

4. The relationship existing between the organism causing infectious abortion in cattle and the organism causing Malta fever in man is reviewed and discussed.

5. The occurrence of Malta fever in the United States is quite infrequent.

6. The recent terminology of the Brucella melitensis group of organisms is discussed.

I wish to express my appreciation to Dr. H. L. Amoss for his many valuable suggestions in the preparation of this paper.

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CURE OF A RETROPERITONEAL AND PERINEPHRITIC INFECTION (ABSCESS) BY INTRAVENOUS INJECTION OF MERCUROCHROME

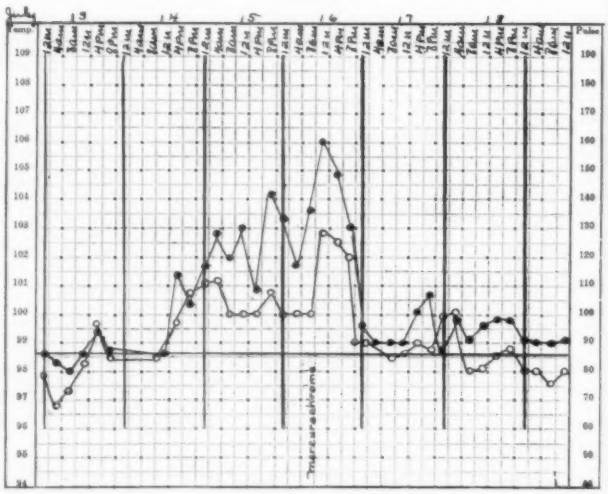
By Hugh H. Young, M. D.

(From the James Buchanan Brady Urological Institute, Johns Hopkins Hospital)

The following case in which the intravenous injection of mercurochrome was successful in causing the prompt subsidence of a retroperitoneal perinephritic infection is sufficiently striking to warrant a brief preliminary report.

The remarkable results which have been obtained with mercurochrome intravenously in septicaemias prompted me to use it in a recent case in which urethral and vesical instrumentation was followed by fever, gradually increasing pain and tenderness in the left side, which finally reached the region above the crest of the ilium and behind the left kidney. Muscle spasm, a temperature of 104°F. and a very toxic state of the patient made the diagnosis of an ascending retroperitoneal infection positive and the necessity for immediate incision for drainage seem imperative. Cultures from the bladder urine showed colon bacilli, and although I knew of no similar case of deep-seated infection that had been treated by intravenous medication, I decided to try mercurochrome in a dosage

CHART 1



of five milligrams per kilogram of body weight (23 c.c. of a 1% aqueous solution per 100 pounds). This treatment was wonderfully successful. After a pronounced reaction accompanied by chill and fever which reached 106°F. two hours later, there was a rapid subsidence of the temperature which reached normal in twelve hours. *Pari passu* there was a rapid disappearance of pain, tenderness, and muscle spasm, and on the following day all signs of toxicity had practically disappeared, and the patient went on to a rapid and uneventful recovery.

CASE REPORT—(Abstract)

J. H. D. (History No. 11532), aged 17, male. Admitted, June 21, 1923.

Diagnosis.—Hypospadias. Operation:—Duplay Plastic to close defect. Perineal drainage. Convalescence uneventful until passage of sounds on 23rd day. This was followed by chill, and fever reaching 101.5°F. in two hours as shown in Chart I.

On the following day the temperature reached 104.5°F., and tenderness, pain and muscle spasm appeared in the left flank. During the day the area of tenderness and muscle spasm increased in the left side and back, but there was no tenderness over the kidney in front.

Diagnosis.—Retroperitoneal infection with formation of abscess following urethral and vesical traumatism. On the third day the temperature was 104°; pulse 100. The

CHART II.

Hour after injection	STOOLS			URINE			VOMITUS			Remarks
	No.	Character	% 220	Amount	% 220	Microscopic	No.	Character	% 220	
1st	0	.....	.....	80 c.c.	1– 50,000	Culture grew bacilli — direct smears negative.	1	Brown with food particles	0	Became nauseated, 20 minutes after injection.
2nd	1	Brown, soft	0	45 c.c.	1– 25,000	No organisms. Few pus cells.	2	Yellow	1–100,000	Sweating profusely. Severe chill. Temp. 106°.
3rd	0	.....	.....	8 c.c.	1– 10,000	No casts.	2	Fluid, yellow	1–100,000	Chill ended. Temp. 103°. Pulse good. Abdominal pain.
4th	1	Fluid, yellow	1– 10,000	10 c.c.	1– 10,000	.....	0	.....	.....	Much more comfortable. Sleeping. Temp. 104°.
5th	0	.....	.....	40 c.c.	1– 10,000	No organisms. No casts.	0	.....	.....	Temp. 105°. Complains of abdominal pain.
6th	1	Fluid, yellow	1– 25,000	.....	.....	.....	0	.....	.....	Temp. 104°. Quite uncomfortable.
7th	0	.....	.....	200 c.c.	1– 50,000	.....	0	.....	.....	Taking fluids well. Temp. 103°. Better.
8th	1	Fluid, yellow	1– 50,000	125 c.c.	1– 75,000	.....	0	.....	.....	Temp. 100.6°. Much better.
9th to 12th	0	.....	.....	760 c.c.	1– 75,000	Cultures show bacilli.	0	.....	.....	Complains of abdominal pain. Temperature normal.
13th to 18th	4	Fluid, yellow tinge	1–100,000	720 c.c.	1–100,000	No casts. Albumin negative. No bacilli.	0	.....	.....	Abdominal pain much better. Muscle spasm disappearing.
18th to 24th	2	Mucous, tinged yellow	1–100,000	400 c.c.	1–100,000	No casts. Smears negative for organism.	0	.....	.....	Temperature has remained 100° or below. Much better.
2nd day	1	Soft	0	1000 c.c.	trace	Negative. Cultures negative.	0	.....	.....	Temp. 99°. Condition improving rapidly.
3rd day	2	Soft	0	1200 c.c.	trace	Negative. Cultures negative.	0	.....	.....	Temp. 98°. Patient feels almost normal. Pain, tenderness and muscle spasm gone.
4th day	1	Soft	0	820 c.c.	0	Negative. Cultures negative.	0	.....	.....	Temperature has remained normal. Patient feels well. Up in chair.

patient was very toxic, and the presence of an extraperitoneal abscess in the left flank was very definite. Immediate operation seemed necessary, but we decided to inject mercurochrome intravenously. Time of injection, 11 A. M., July 16; dose 27 c.c. of a 1% aqueous solution—5.4 mg. per kilo of body weight.

The above chart, with its careful colorimetric tests, which were made at my request by Dr. E. C. Shaw, shows the remarkable rapidity with which mercurochrome appears in fairly high percentage in the urine, stools and stomach contents (vomitus). Within four hours mercurochrome was present in both urine and stools in a strength of 1 to 10,000. As shown by Miss J. H. Hill, a solution of 1 to 5,000 kills B. coli in urine and salt solution in one hour, and a solution of 1 to 10,000 kills staphylococci in one hour. It remained in this strength apparently for at least three hours, after which it gradually diminished; but twenty-four hours later was still at a strength of 1 to 100,000.

The improvement of the patient, after recovering from the primary reaction in which the temperature rose to 106°F. was most gratifying—the subsidence of pain, tenderness, muscle spasm and mass in the left flank and back being indeed remarkable. The patient was discharged on the sixth day after the intravenous injection, in excellent condition. Nov. 1, 1923 (4 months later), Letter: patient well.

One cannot, of course, draw positive or general conclusions from a single instance, but coming as it does after definite laboratory proof that mercurochrome, intravenously administered, will sterilize the blood and urine (Hill and Colston) in animals infected with the bacillus coli or other bacteria, and after our case of a patient, almost moribund with colon bacillus septicaemia who was promptly cured by 34 c.c. of a 1% solution, and cases of pyelitis which Colston and I have sterilized in this way, it makes me feel confident that the effect of the drug in this case was very definitely curative and that the procedure is worthy of further clinical experimentation. I wish to express my thanks to Dr. E. C. Shaw and to Miss J. H. Hill for valuable assistance.

Conclusions

In the case, here recorded, it is shown that a colorimetric method can be used to determine the strength in which mercurochrome, after intravenous injection, may appear in urine, stools and vomitus. A strength of 1 to 10,000 was maintained in the voided urine for three hours, and also in stools. The immediate sharp febrile reaction (to 106°F.) followed by a rapid fall to normal, and a disappearance of definite symptoms and signs of a retroperitoneal abscess, seem to verify previous animal experiments which show a pronounced general and local germicidal effect from intravenous injections of mercurochrome.

CHRONIC ARTHRITIS IN THE ADULT, ASSOCIATED WITH SPLENOMEGALY AND LEUCOPENIA

A REPORT OF FIVE CASES OF AN UNUSUAL CLINICAL SYNDROME

By A. R. Felty, M. D.

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The occurrence of slight splenic enlargement associated with the arthritides has occasionally been noted by various writers. McCrae found it to be present in a small group of his studied cases (among which were included cases of deforming arthritis of children); but he states that splenomegaly is much more common in acute arthritis than in the chronic type. So, too, leucopenia may occasionally be present in arthritis, though it is not commonly believed that the white cell count of the blood is characteristic or of particular significance, since variations above and below the normal are of about equal occurrence. However, the association of all three conditions is a rather more unusual circumstance, and a recent observation of such a case has led us to search the hospital records for similar pictures. Five cases, of striking similarity in many respects, have been collected, presenting a syndrome which is not only of interest by itself, but offers certain diagnostic difficulties which will subsequently be mentioned.

Report of Cases

Case 1.—(Medical No. 49918.) A white man of 46, an American, was admitted to the hospital August 12, 1923, with the complaint of generalized aching in the muscles and joints, of three years' duration. There was no history of arthritis in his family. He gave a personal history of several mild short attacks of malaria, the last attack ten years before admission. Three years before coming to us he began to have attacks of arthralgia, the aching being more or less generalized, but worse in his ankles and wrists, which sometimes were swollen. There were intervals, however, during which there was almost complete freedom from symptoms. Since the onset he had lost about 40 pounds; and in recent months his appetite had been poor, with occasional nausea after meals.

Physical Examination.—A sparely nourished man, who has evidently lost considerable weight. Slight pallor. Exposed surfaces deeply pigmented. Axillary, epitrochlear, and inguinal glands slightly enlarged and firm. Pupils and eye-grounds normal. Throat clear. Pyorrhea about the few remaining teeth. Heart and lungs clear. Pulse regular, no radial thickening. B. P. 115/75. Abdomen soft, symmetrical, not tender. Spleen felt as a firm, rounded mass extending to the level of

the umbilicus, 6 cm. below the costal margin in the mammillary line. A thin, soft liver edge comes just to the costal margin. A little swelling and tenderness of the 2nd and 3rd metacarpo-phalangeal joints of both hands, and slight fusiform enlargement of the interphalangeal joints. Some soreness on passive motion of the fingers, elbows, shoulders, ankles, and toes, but no objective changes except in the fingers. Slight grating in both knees. Left elbow somewhat limited in mobility, apparently from voluntary spasm. There is general soreness of the muscles of the extremities. No local heat nor muscular atrophy. Spine negative. Slight hyperreflexia.

Blood Examinations.

8/14/23. R. B. C. 3,750,000; Hb. 70%; W. B. C. 2680.
Differential formula.
P. M. N. 26%; P. M. E. 2%; S. M. 54%; L. M. and Trans. 18%.
Slight diffuse and punctate basophilia.

8/27/23. W. B. C. 3000.

9/ 2/23. W. B. C. 2800; Hb. 80%.
P. M. N. 34%; P. M. E. 2%; S. M. 54%; L. M. and Trans. 9%.

9/ 7/23. W. B. C. 3000.
P. M. N. 47%; P. M. E. 4%; S. M. 30%; L. M. and Trans. 9%.
R. B. C. show diffuse and punctate basophilia.
Platelets, 330,000.
Bleeding time, 2½ minutes.
Coagulation time (Howell method), 10 minutes.
Reticulated R. B. C., 0.8%.
Fragility of R. B. C.
Hemolysis begins at..... 0.46% NaCl
Hemolysis of control begins at.. 0.44% NaCl
Hemolysis complete at..... 0.32% NaCl
Hemolysis of control complete at 0.34% NaCl

Urine.—Negative except for slightly positive urobilin test obtained on three occasions.

Test Meal.—No free acid present.

Stool.—Negative.

Wassermann reaction.—Negative.

8/17/23. Blood culture negative.

9/ 7/23. Blood chemistry—
Non-protein Nitrogen ...28 mgm. per 100 c.c.
Sugar .....85 mgm. per 100 c.c.
Uric acid ..... 3.2 mgm. per 100 c.c.

X-ray of chest.—Lungs clear. Heart and aorta normal.

X-ray of joints.—Knee and elbow show practically no bone or joint changes.

Pathological report on section of excised epitrochlear gland—"Chronic lymphadenitis."

Examinations by the laryngologist and dentist were negative.

Temp. 99°—100.5° (rectal) for two weeks; normal thereafter until discharge.

Summary of Case.—White man, 46 years old, with a history of joint pains for three years. Undernutrition. Pigmentation of exposed parts. Splenomegaly. Objective changes in joints which, however, are not proportionate to the symptoms. Slight glandular enlargement. Slight anemia and frank persistent leucopenia. Slight urobilinuria. Gastric anacidity.

Impressions of case (by different observers) (1) Syndrome in adult comparable to that of Still's disease; (2) Possible Banti's disease associated with chronic arthritis.

CASE 2.—(Med. No. 19,574.) A Lithuanian woman, 45 years of age, sought admission to the clinic on April 16, 1906, on account of generalized aching in her joints. Family history irrelevant. Her husband had died of phthisis in 1899. Her own health had always been fairly good, though she had had typhoid fever, and occasional sore throats. Of late her appetite had been poor. Menopause six months before admission. Occasional cough in winter. She had lost about six pounds in the past four or five months. Her present illness began six years before admission with pains in her joints, which had been present more or less constantly since then—varying, however, in severity and distribution. About four months before admission she was confined to bed for three weeks with fever, and swollen, painful feet.

Physical Examination.—The patient has a cachectic sallow appearance, is undernourished, and somewhat anemic. Light brown pigmentation over abdomen, on flexor surfaces of arms and in axillae. Conjunctival inflammation characteristic of trachoma. Teeth in bad condition. The cervical, axillary, and epitrochlear nodes are slightly enlarged and firm. Heart normal. Lungs negative except for slight emphysema. Visible peristalsis present. Spleen extends 13.5 cm. diagonally below the costal margin, and 5 cm. below the level of the umbilicus, measuring 8 cm. in width at the rib edge. A notch is felt. Liver not enlarged. Slight crepitus felt in the shoulders and right knee, although mobility is not restricted. Both wrists show limitation of motion, but no pain nor swelling. Left knee is held flexed and the joint is tender. Fingers and toes abducted and somewhat flexed.

Blood.—R. B. C. 4,624,000. Hb. 72%. W. B. C. 1000.

Urine.—Negative (urobilin not tested for).

No fever during stay on ward. Patient discharged after five days on account of trachoma.

Summary of Case.—Woman of 45, who has had symptoms of arthritis for six years. Undernutrition and mild anemia. Brownish pigmentation of skin. Great enlargement of spleen. Slight arthritic changes in joints. Very marked leucopenia.

Diagnosis.—Arthritis deformans; splenomegaly; trachoma.

CASE 3.—(Med. No. 35,980.) The patient was a white man, aged 65, an American, who was admitted to the hospital April 24, 1916, complaining of articular aching of the extremities. The affection had had a rather sudden onset eight months before with swelling, redness, and pain in one ankle, with subsequent rapid involvement of all the other joints of the extremities—especially the ankles, knees, wrists, and elbows. The arthralgia persisted with exacerbations and remissions. There was an indefinite history of a somewhat similar attack fifteen years before, which had left no residuum after its disappearance. The patient had lost forty pounds since the onset of the present illness. The past and family history were non-contributory.

Physical Examination.—Poorly nourished man, who has evidently lost considerable weight. Exposed surfaces of skin show brownish pigmentation. Fundi normal. No general glandular enlargement. Teeth in poor condition. Throat clear. Lungs normal except for slight emphysema. Heart normal. Blood pressure 140/80. Liver not palpable. A firm spleen reaches to the level of the umbilicus. Slight limitation of mobility of the shoulders. Periarticular swelling of both elbows, which are held in partial flexion, with slight limitation of motion. Slight thickening of both wrists and metacarpo-phalangeal joints, with normal mobility. No Heberden's nodes. There is a small collection of fluid in both knee-joints, which are limited in mobility. Ankles somewhat swollen, but freely movable. Spine normal. Rectal examination negative.

Blood Examination.—R. B. C. 4,800,000. Hb. 80%. W. B. C. 2720. Differential, P. M. N. 74%. S. M. 19%. L. M. and Tr. 5%.

Urine.—Negative.

Stool.—Normal.

Wassermann reaction.—Negative.

X-ray Examination.—"Infectious arthritis with some slight bone change."

Special Examination of the nose and throat revealed nothing abnormal.

The patient was afebrile throughout his six weeks' stay in the hospital.

Summary of Case.—White man, 65 years of age, with symptoms of arthritis in arms and legs for a period of eight months. Undernutrition. Pigmentation of exposed surfaces. Splenomegaly. Leucopenia. Slight periarticular swelling, pain on motion, and slight limitation of mobility of some of the joints of the extremities.

Impression of case: Chronic infectious arthritis; splenomegaly. Possibility of Banti's disease suggested by the enlarged spleen and leucopenia.

CASE 4.—A white woman, 50 years of age, was admitted to the hospital October 31, 1914, complaining of pains in her joints, of about ten years' duration. Her general health prior to this had been good, and her past history, except for an attack of pleurisy 25 years before admission, was singularly negative. She had never had malaria. Her two children were living and well. The menopause occurred in July, 1914. Since 1908, when she weighed 190 pounds, she had gradually lost 65 pounds to her present weight of 125 pounds. In 1904 her present symptoms began with an attack of pain and swelling in both ankles which confined her to bed for a few days. After the subsidence of the acute attack she continued for five years to have slight aching with swelling of her ankles, and during this period she had three or four attacks similar to, but less severe than, the first. The ankle joints had become somewhat stiff and continued to give pain. In the past five years other joints had become involved in succession—the metacarpo-phalangeals, the wrists, knees, toes, and elbows. The swelling and pain in the joints had been variable, at times acute with reddened skin and slight local heat, and at no time had there been complete subsidence of the symptoms. For the past six months the articular pains had been more generalized and more severe, especially in the ankles.

Physical Examination.—The patient is distinctly undernourished and has obviously lost weight. Sallow pigmentation of exposed parts, especially about the eyes. Fundi normal. Marked pyorrhea. Tonsils adherent to pillars. No lymph-node enlargement. Heart and lungs clear. Radial vessels soft. (The spleen comes to the costal margin.) Liver edge not felt. Symmetrical, soft, painless swelling of the metacarpophalangeal joints of both hands, with no redness or local heat. Slight ulnar deflection of the fingers with slight limitation of motion. No Heberden's nodes. Small muscles of hands a little atrophied. Right elbow and both knees slightly limited in mobility, but are neither red nor tender. No crepitus in any of the joints. Pelvic examination, negative.

Blood Examination.

10/31/14. R. B. C. 4,528,000. Hb. 70%. W. B. C. 2080. P. M. N. 46%. P. M. E. 11%. P. M. B. 0.5%. S. M. 24%. L. M. and Tr. 18%.

11/23/14. R. B. C. 4,000,000. Hb. 74%. W. B. C. 2440. P. M. N. 41%. P. M. E. 11%. P. M. B. 1%. S. M. 20%. L. M. and Tr. 27%.

11/23/14. R. B. C. 5,228,000. Hb. 80%. W. B. C. 2040.

12/ 1/14. R. B. C. 5,000,000. Hb. 80%. W. B. C. 2000.

12/ 6/14. W. B. C. 2,240. P. M. N. 25%. P. M. E. 13%. P. M. B. 1%. S. M. 38%. L. M. and Tr. 23%.

12/ 9/14. R. B. C. 4,700,000. Hb. 79%. W. B. C. 2040. P. M. N. 37%. P. M. E. 12%. P. M. B. 1%. S. M. 28%. L. M. and Tr. 22%.

1/11/14. R. B. C. 4,500,000. Hb. 76%. W. B. C. 1760.

Urine.—Negative.

Stool.—Negative.

Wassermann.—Negative. Gonococcus complement fixation test negative.

Vaginal Smears.—Negative.

X-ray of wrist and ankle joints showed chronic infectious arthritis.

X-ray of sinuses was negative.

Special nose and throat examination was essentially negative.

The patient ran a low irregular fever which subsided after six days.

Summary of Case.—A white woman, 50 years of age, with symptoms of arthritis of the extremities for ten years, with exacerbations and remissions but never complete subsidence of articular pain. Marked undernutrition. Pigmentation of the exposed surfaces of the skin. Splenomegaly. Slight objective changes in many joints of both arms and legs. Slight secondary anemia and frank leucopenia.

Impression of case: Chronic infectious arthritis; splenomegaly.

CASE 5.—(Med. No. 30,042.) A white man, aged 51 years, an American, came to the hospital December 3, 1912, complaining of rheumatic pains of two and one-half years' duration. Prior to this he had always been in good health, with no serious illnesses. Acute urethritis once 25 years before admission. He had lost 40 pounds in the last two years. The gradual onset of his arthritic symptoms dated back to April, 1910, when he began to have aching pains and swelling of the left foot. A few weeks later both wrists and shoulders became affected. In September, 1910, the left and then the right knee became hot, swollen and painful, but after a short time there was complete subsidence of symptoms in both knee-joints for eighteen months. A year before admission the left hip became painful. For the past two months there had been a flare-up of symptoms in all the joints (knees, hips, wrists, fingers, and shoulders).

Physical Examination.—The patient shows poor nutrition, with evident loss of weight, and poor muscular development. Pigmentation similar to tanning on backs of hands and exposed parts of head. Labial herpes. Lips rather pale. Throat clear. Axillary, inguinal, and epitrochlears a little enlarged. Persistent fine moist râles at right apex. Heart negative. Blood pressure 120/80. Abdomen scaphoid; the spleen extends below the costal margin. Polyarthritis involving especially the knees, hips, fingers, wrists, and elbows with slight swelling, periarticular infiltration of the soft parts, and limitation of motion, partly voluntary, partly organic. Practically all the joints of the extremities are sore. Crepitus in knees, and wrists, with slight muscular atrophy.

Blood Examination.—R. B. C. 4,304,000. Hb. 85%. W. B. C. 4200.

Sputum Examination.—Negative for tubercle bacilli (numerous examinations).

Urine.—Negative.

Wassermann reaction.—Negative.

X-ray of left knee and foot. Slight infectious arthritis of knee; foot negative.

X-ray of chest.—Infiltration of both apices.

Temperature remained normal throughout the patient's stay in the hospital.

Summary of Case.—White man, 51 years of age, with a history of generalized articular pains for two and a half years, and loss of forty pounds during this time. Undernutrition. Slight enlargement of lymph-nodes. Tan-like pigmentation over exposed parts. Râles at right apex. Enlarged spleen. Arthritis of numerous joints of the extremities. Leucopenia.

Impression of case: Chronic infectious arthritis; pulmonary tuberculosis (inactive); splenomegaly.

ANALYSIS AND DISCUSSION

Certain features of these five cases are outstanding and worthy of comment. The syndrome occurred in individuals of middle age (45-65), the average being 50 years. All the patients gave a history of marked loss of weight since the onset of symptoms (from 40 to 65 pounds in four out of five cases), and all were undernourished. The arthritic process is distinctly chronic, and all but one of the patients had symptoms of over two years' standing, the average duration being four and one-half years. With either an acute or gradual onset, there develops more or less generalized articular aching in the extremities, mild but persistent, and accentuated by occasional acute exacerbations. In striking contrast to the prolonged course of the disease, and the ubiquitous distribution of the pain which is the presenting symptom of all the patients, the objective findings both by physical examination and roentgenographic study are neither wide-spread nor indicative of a very damaging or destructive process. Indeed, one is rather impressed by the relatively benign nature of the involvement when compared with the typical chronic deforming arthritis of equal duration and extent.

In every case the spleen was palpably enlarged—just below the costal margin in two cases; in two others, extending to the level of the umbilicus; and in the fifth case reaching a point 5 cm. below this level. The spleens were firm and not tender. None of the patients had symptoms referable to the splenic tumor. No abnormalities in the size or consistence of the liver were noted. The lymph glands were not enlarged in two cases; but in the other three, the axillary, inguinal, and epitrochlear nodes showed definite enlargement. In every instance there was noted a yellowish-brown pigmentation of the skin, which in four cases was confined to the exposed surfaces, but in the fifth had a wider distribution over the abdomen, axillae, and flexor surfaces of the arms. None of the patients had hypertension. The heart, lungs, and eyegrounds were essentially normal.

In all cases save one there was a slight secondary anemia—the red cells varying from 3,750,000 to 4,800,000; the hemoglobin from 70-80%. The average red count was 4,400,000, and the hemoglobin 75%, giving a color index of 0.85. More striking was the leucopenia, which was a distinctive feature in every case. The leucocyte counts varied from 1000 to 4200, in four cases being below 3000. The differential formula was not characteristic. The urine was normal except for the one case in which urobilin was found to be present. Stool examinations were negative. None of the patients showed any evidence of lues either serologically or on general examination. Three patients were afebrile during their hospital stay; two ran a low fever which subsided after one to two weeks.

The question immediately arises as to a proper classification, or at least an explanation of this unusual clinical syndrome. There are but two likely possibilities:

1. The several features are manifestations of one pathological process, caused by a noxa which simultaneously affects the joints, the spleen, and the blood leucocytes (and in three of the five cases the lymph glands). On such a basis, the picture might be comparable to that occasionally seen in young children, and first described by Still—a syndrome of chronic arthritis, splenomegaly, and general gland enlargement which has since been designated as Still's disease. But whereas Still's disease occurs only in the young, usually before the second dentition, this disease is distinctly one of middle life (45-65 years). So, too, the leucocyte count in Still's disease is not characteristic, and, if altered at all, is more commonly increased, whereas in the present series of cases there is a constant and very striking leucopenia.

2. The syndrome is merely the confusion of two separate clinical entities, occurring coincidently in the same individual. Taken separately, without regard for the arthritis, the enlarged spleen, with leucopenia and slight secondary anemia, the slight pigmentation of the skin, and the presence in the urine of urobilin, in the one case in which this was sought for—these findings are typical features of early Banti's disease. This possibility was indeed broached in two of the cases. Joint changes, however, are not a part of the Banti syndrome, and Eppinger, in his exhaustive discussion of this disease, fails to mention any observations of its occurrence. One would need to assume, then, that the arthritis is quite independent of the rest of the complex. However, on the law of probability alone, such a coincidence seems highly unlikely, and this view would violate a fundamental rule of diagnostics—that a symptom-complex should, if possible, be explained on the basis of one pathological process rather than on the assumption of the existence of two or more. In one case, to be sure, the history of several short malarial attacks many years ago might possibly explain the existing splenomegaly (though not the urobilinuria nor the leucopenia). In the other cases there was no pre-existing disease recognized to explain the findings. In short, one is more or less forced to the conclusion that this syndrome is a distinct clinical entity, of which the outstanding symptoms are those related to the joints, and

the outstanding signs are the enlarged spleen and the blood picture.

SUMMARY

Five cases, strikingly similar in their essential features, are described, presenting an unusual but unmistakable clinical picture, characterized by arthritis, splenomegaly, and leucopenia. The etiology is entirely obscure, though the various findings seem best accounted for as manifestations of a single disease process.

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RESULTS OF THE INJECTION OF ENCEPHALITIC SPINAL FLUID INTO RABBITS

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Experimental encephalitis and experimental herpes, now so closely associated and believed by some to be identical, were in the beginning widely separated in the minds of experimental pathologists.

Loewe and Straus,[1] in 1920, reported the transfer of epidemic encephalitis to rabbits by the intracerebral injection of filtered nasopharyngeal washings and of spinal fluid from human cases. Levaditi and Harvier,[2] Kling,[3] and Thalhimer [4] soon confirmed this report.

In a totally separate line of investigation Loewenstein [5] produced keratitis in rabbits by applying to the scarified cornea the contents of herpetic vesicles. Some of the rabbits died of convulsions suggesting that the virus had invaded the central nervous system.

Doerr with Vöchting [6] and with Schnabel,[7] injected the vesicular contents intracerebrally into rabbits and found that a diffuse encephalitis was provoked by the virus. Doerr noted the close similarity between herpetic virus and the virus of epidemic encephalitis, and Levaditi from a thorough experimental study believed that the two viruses are identical and adduced immunological evidence to substantiate his views. Kling, on the other hand, from immunological studies does not agree. In spite of the great amount of work on the subject of the identity of herpes and encephalitis there still remains some doubt in the minds of many. One of the questions to be decided relates to the authenticity of the so-called encephalitic strain.

There is no doubt that some sort of virus supposed to have come from human encephalitis has been propagated among rabbits, but it cannot be said definitely that this strain represents the true encephalitic virus. There are at least two possibilities, (a) that some virus peculiar to rabbits and producing the lesions indistinguishable from encephalitis, as described by Oliver [8] and Wright,[9] has been propagated, and (b) that the so-called encephalitic virus is really a human herpetic strain which entered the spinal fluid on account of an increased permeability of the meningeal choroidal complex.[10] Netter and McNalty report a very low incidence of herpes simplex in epidemic encephalitis.

Efforts to transmit spontaneous rabbit encephalitis during the past year have been in vain. Likewise, attempts to isolate a strain of herpes from the spinal fluid of a few patients with marked labial herpes but without signs of encephalitis have yielded negative results.

EXPERIMENTAL

Fifteen young rabbits were given intracerebral injections of 0.4 c.c. spinal fluid withdrawn from two patients on the second and third days after the development of labial herpes. Laboratory examination of these fluids was entirely negative. None of the rabbits injected showed any signs of herpes encephalitis.

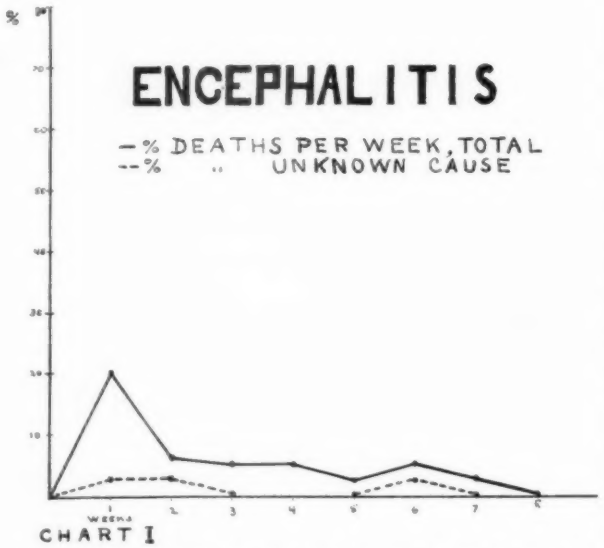

CHART I

40 inoculated in all; 20 died in 2 months, 50%; 7 died from coccidiosis; 5 died from pneumonia; 2 died from inoculation; 1 died from fractured spine; 1 died from diarrhea; 1 died from brain abscess; 3 died from unknown cause.

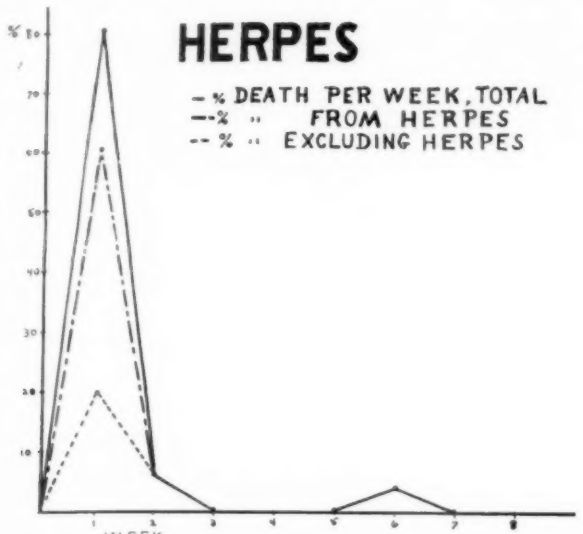

CHART II

24 inoculated in all; 22 died in 2 months, 92%; 14 died from herpes; 4 died from pneumonia; 3 died from unknown cause; 1 died from fractured spine; 2 survived.

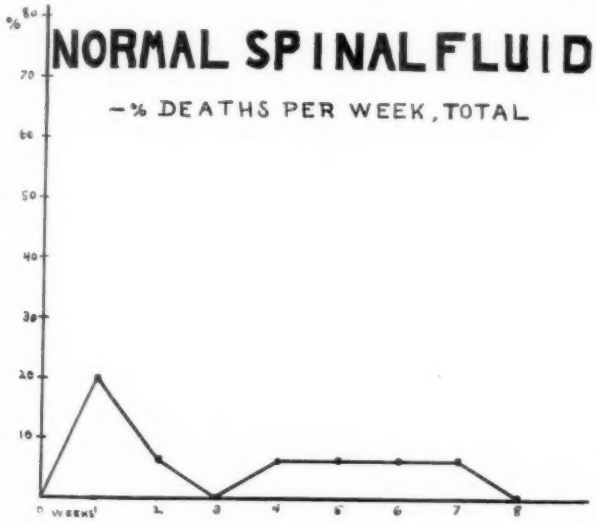

CHART III

14 inoculated in all; 8 died in 2 months, 57%; 3 died from pneumonia; 1 died from slobbers; 2 died from diarrhea; 1 died from unknown cause.

ATTEMPTS TO TRANSMIT EPIDEMIC ENCEPHALITIS TO RABBITS

During the past year about 400 rabbits were used in attempts to transmit various infections of the central nervous system. The present report deals with a small, carefully controlled, parallel series of rabbits injected with material from herpes and from encephalitis.

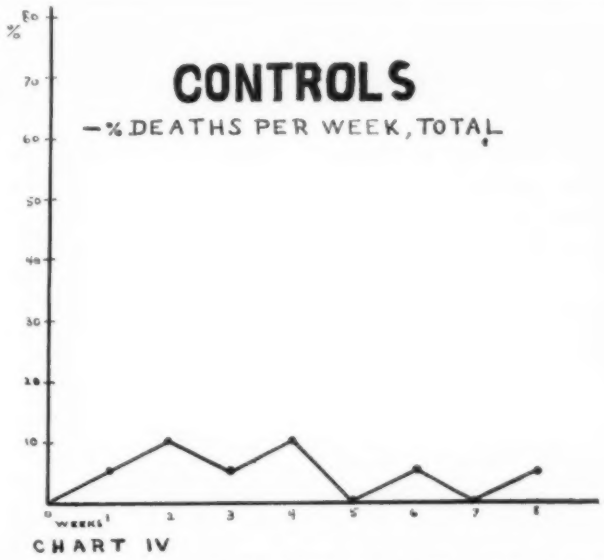

CHART IV

19 total number controls; 8 died in 2 months, 42%; 4 died from pneumonia; 1 died from coccidiosis; 1 died from peritonitis; 2 died from unknown cause.

Technique.—The material used was fresh spinal fluid obtained from thirteen patients ten of whom were in the acute stage of epidemic encephalitis, one was undergoing a recrudescence of symptoms and two others were in the chronic stage presenting a well marked Parkinson syndrome. Suspensions of brain substance from two patients who had died six and ten days after onset were also employed.

Only young rabbits, about one kg. in weight, such as were found most susceptible to experimental herpes, were employed. All inoculations were made directly into the left cerebral hemisphere with a fine needle passed through a thin area in the temporal bone. Where spinal fluid was used, several cubic centimeters were also injected intravenously. A series of forty rabbits were inoculated in this group, thirty-six with spinal fluid and four with brain substance (see Chart I).

Controls.—As controls three other series of rabbits were used for comparison. The rabbits in the second series were inoculated with fluid aspirated from vesicles of several different cases of herpes labialis (see Chart II). A third series received injections of normal spinal fluid (Chart III) and a fourth series received no inoculation but were kept as normal controls. All rabbits were observed for two months or longer. Careful routine autopsies were performed and sections from the forebrain, midbrain, and hindbrain were cut in paraffin and stained with hematoxylin and eosin.

Results.—Chart I shows the results of the first series of experiments in which spinal fluid or brain suspensions from cases of encephalitis were used. Twenty of forty rabbits inoculated, died in two months, and seventeen of

these showed various adventitial conditions at autopsy sufficient to cause death. During the first week after inoculation the mortality was twenty per cent. Afterwards an average of five per cent died each week.

Results of the second series of inoculation with herpes virus are shown in Chart II. The total mortality was ninety-two per cent in two months. During the first week seventy-nine per cent died, and about sixty per cent of these showed typical symptoms of herpes encephalitis. The incubation period in these later cases was very definitely from three to six days. Three rabbits in this series died without any typical symptoms and showed no cause for death at autopsy.

Of fourteen rabbits injected intercerebrally with normal spinal fluid, eight died of various conditions tabulated in Chart III, a total mortality of over fifty per cent. The high mortality for the first week could be due only to the direct or indirect effect of the inoculation. After that the mortality averaged seven per cent per week which is quite comparable with that found in the fourth series of control rabbits as shown in Chart IV. One rabbit showed no demonstrable cause of death at autopsy.

Thus it seems clear that under the experimental conditions the average death rate per week was about five per cent for normal rabbits, and that the injection of even non-infectious material, i.e., normal spinal fluid, causes a temporary increase in mortality up to twenty per cent for the first week. The mortality per cent curves for the first and third series are essentially identical, and show merely the average mortality and the temporary increase of mortality produced by the injection. The mortality curve for the second series shows unmistakably the effect of an infection with a definite latent period, and if the cases which showed symptoms of herpes encephalitis are subtracted from the total, the mortality curve is seen to be identical with that of the first and third series. The small percentage of deaths unexplained by autopsy is approximately the same in all four series.

Symptoms.—Clinically, the rabbits in Series I (encephalitic material) showed nothing characteristic or constant. Some had terminal convulsions, some rushed madly about as if in delirium and some became prostrate, but none, except those with fractured spines, showed true paralysis. Control rabbits likewise exhibited the same symptoms. However, the symptoms of experimental herpes encephalitis were highly characteristic, so that it was easy to recognize the condition at sight. The first definite sign was usually slight salivation and restlessness, followed rapidly by generalized tremor, retraction of the head, gritting of the teeth, more profuse salivation and generalized convulsions which started in the limbs opposite the hemisphere injected. There was retention of urine and death occurred eight to ten hours after symptoms appeared.

Microscopic Examination of Brain Tissues.—About fifteen per cent of the brains of the rabbits in the first series showed small focal areas of round cell accumulation principally in the cortex and some slight perivascular infiltration such as have been frequently described. Uninoculated control rabbits showed similar lesions indistinguishable from the lesions found in the rabbits of the first series in about the same percentage. Without exception the rabbits of Series II which died with clinical symptoms of herpes encephalitis showed wide-spread inflammatory changes, which were very similar to the lesions described above, but differed in showing a more diffuse distribution and more frequent meningeal involvement.

Discussion and Summary

These results are reported in order to call attention more especially to the fact that after subdural injection, in some instances, a definite mortality curve is to be expected, and may lead to false conclusions as to the presence of a virus in the fluid inoculated. In the series reported it appeared at one time that a strain had been established, but later events revealed that this suspicion was incorrect. A comparison of Charts I, III and IV show that the mortality in the encephalitic series is almost identical with that resulting from the injection of normal spinal fluids, and of isotonic salt solution, and is in striking contrast to the results obtained in the series injected with herpetic virus (Chart II).

Thus, the injection of spinal fluids from thirteen cases of encephalitis and of fresh brain suspensions of three cases, did not furnish any evidence that these specimens contained a virus capable of producing experimental encephalitis in rabbits. The low mortality from unknown cause shown by the broken line in Chart I needs a word of explanation. No cause of death was found, but the injection of the fresh brain into five rabbits in each instance did not in any case yield experimental encephalitis.

The negative results in the whole series of encephalitic rabbits contrasts strongly with the positive results of Straus and Loewe (70%). No explanation is offered for this discrepancy. Certainly ample opportunity for the propagation of the virus was given, as at least five rabbits received a part of each fluid or brain suspension tested.

Finally, from three cases of marked herpes simplex, the spinal fluid did not yield, under the conditions of the experiment, the herpetic virus.

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FURTHER STUDIES ON BLOOD GROUPING

I. THE ANTIGENIC PROPERTIES OF TWO TYPES OF "GROUP II" ERYTHROCYTES*

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In an earlier communication [1] evidence was presented of the existence of more than four isoagglutinin groups in human blood. The evidence consisted of personal observations and experiments with the bloods of persons who obviously formed exceptions to the conventional "rule of four." Scrutiny of the published reports of other workers revealed the fact that such exceptions had been encountered before, without, however, attracting attention to their real significance. The results as summarized in the report of our earlier investigation were as follows:

1. The methods generally employed for blood grouping are based upon the assumption that there are only four isoagglutinin groups, and that the blood of every human being belongs in one of these groups. The tests based on this assumption are so devised as to cause each blood tested to fall into one or other of these four groups, thus serving to perpetuate a belief which no one has seriously questioned.

2. We have presented evidence that the popular belief concerning the existence of four and only four isoagglutinin groups is incorrect.

3. The presence of only two isoagglutinins and two isoagglutinogens is inadequate to explain our findings.

4. By direct tests and by absorption experiments, the existence of a third isoagglutinin and a third isoagglutinogen has been demonstrated. Although recognizing the possibility that there may be still other isoagglutinins and isoagglutinogens in human blood, we have found nothing thus far to indicate their existence; our observations have shown, however, that there are *at least* three of each.

5. With three isoagglutinins and three isoagglutinogens there are 27 combinations biologically possible.

6. We have found bloods corresponding to 8 of these combinations, and by citations from the literature have indicated the probable existence of a number of others.

7. Some of these combinations are undoubtedly more common than others; some may be excessively rare; others may not occur at all; it may not be possible to prove the existence of all of them.

8. We believe that these findings have a bearing of practical importance upon transfusion and furnish a plausible explanation for some of the hitherto unexplained post-transfusion reactions.

9. Allowance has been made for the possible occurrence of autoagglutination, which we believe has not been a complicating factor in our results.

10. The grouping of bloods should be determined on a basis of their agglutinin-agglutinogen content.

11. Only bloods with the same agglutinin-agglutinogen content belong to the same group.

12. Methods for the classification of blood groups have been suggested.

That portion of the work quoted above which concerns us at present was the discovery of two distinct types of blood both of which are ordinarily classified as belonging to "Group II." * To the usual tests these two bloods reacted in just the same way; even in complete cross-agglutination tests with members of the other three accepted groups their reactions seemed identical; but by other measures it was possible to distinguish between them quite definitely.

By absorption tests with the sera of certain members of "Group III" and "Group IV" it was shown that the red cells of one type contained an additional agglutinogen not present in the red cells of the other type. This finding was confirmed by the action of untreated serum from an unusual blood, which was capable of agglutinating the cells containing the additional agglutinogen but not those of the other type. From these observations we were led to assume that the formulae which would explain the behavior of these two types of "Group II" blood were as follows:

Blood	Serum contains agglutinin	R. B. C. contain agglutinogen
Group II (H.)	*A*	*b*
Group II (D. J.)	*A*	*bc*

Our study showed that neither of these types was unusual and suggested (1) that the type represented by the formula *A*—*b* was commoner than that represented by *A*—*bc*, and (2) that their relative proportion within "Group II" was about 4 to 1. Subsequent observations,

* Submitted for publication, Sept. 24, 1923.

* We found two types at that time; there may be several others included in "Group II."

however, have led us to question the accuracy of our earlier impression concerning the relative frequency with which these two types are encountered within "Group II." *

* The statements in our previous report [1] bearing on this point were as follows:

"We cannot state with absolute assurance which is the commoner of these two types, as it is easy to be misled from a small series. Absorption tests to determine this point have not been carried out extensively. We have been able, however, to test this point more directly. Red blood cells from 142 patients in the wards of the hospital were tested with the serum of C. T., and those from 13 were agglutinated. Cross-agglutination tests were then made with these 13 bloods, the blood of C. T., Group II (H), Group II (D. J.), and blood from tested representatives of Group I, Group III and Group IV. The results of these cross-agglutination tests are shown in Table XX.

"Table XX shows that the bloods of each of these 13 patients reacted to the members of the 4 known groups like members of Group II, but that their behavior with the blood of C. T. was like that of Group II (D. J.), rather than like that of Group II (H.). We were unable to continue this work until 12 days later, by which time all but 4 of the 13 patients had left the hospital. The serum of C. T. again agglutinated the cells of these four patients and the cells of Group II (D. J.). After absorption of C. T. serum with the cells of D. J., it agglutinated the cells of none of these 5 persons.

"Unfortunately we did not group all of the 142 bloods used in the experiment, but only those of the 13 agglutinated by the serum of C. T., all of which behaved like Group II. In the total number we might have expected to find about 40 per cent or 56 members of Group II. From this it would seem that the formula $A—b$ is commoner than $A—bc$."

* * *

"In Section III, 4, we have shown that bloods ordinarily assigned to Group II but having the formula $A—bc$ are not very infrequent, as we found 13 in testing the bloods of 142 persons (9 per cent). We may assume that about 40 per cent of the 142 bloods would be classified under Group II, and so may assume that in every 4 or 5 bloods considered as belonging to this group, one of them is likely to have the formula $A—bc$."

Our reason for questioning these statements is that in our later work we have found bloods with the formula $A—bc$ much more often than would be expected from the proportion given above. The explanation is, we believe, to be found in the peculiarities of the agglutinating serum (C. T.) used in the differentiation just quoted. This serum contained, in addition to isoagglutinin C, an autoagglutinin which was active not merely against the cells of C. T. but against those of members of all the 4 accepted groups as well. The presence of the autoagglutinin was manifest only at low temperatures, but this rendered it imperative to carry out all of our tests for the presence of isoagglutinin C in the serum C. T. at 37°C.; this is not the optimum temperature for isoagglutination reactions, as will be pointed out in a following communication. Had it been practicable to make the tests with this particular serum at a lower temperature or had we used an absorbed "Group IV" serum for the purpose of determining the relative frequency of these two types of "Group II" blood, it is probable that our results would have been quite different.

The present communication deals with these two types of "Group II" blood, which will be referred to either as H. and D. J. or by the formulae given above.

In a very interesting paper, Hooker and Anderson [2] reported that they had been able to produce specific agglutinating sera for the four accepted groups of human erythrocytes by immunization of rabbits. Not every attempt was successful, as they recorded 9 failures in the 14 rabbits injected. They assumed the presence of an antigen common to the four groups of human red cells and also that the specific antigenic substances or isoagglutinogens in the red cells might be capable of acting as heteroagglutinogens in calling forth the production of specific heteroagglutinins in rabbits. Both of these assumptions were borne out by the results of their successful experiments. In several instances they were able to absorb from the immune serum the agglutinin produced by the antigen common to all four groups of cells and retain that which was produced by the specific antigenic action of the particular type of cells used for immunization.

Object

This report of the artificial production of group-specific haemagglutinating sera suggested to us an additional method of securing information calculated to confirm or disprove our conclusions concerning the agglutinogen content of our two types of "Group II" blood. If the red cells of D. J. really contain two isoagglutinogens (b and c) and the red cells of H. only one (b), by the immunization of rabbits it might be possible to secure sera which would distinguish between them. Following the reasoning of Hooker and Anderson, let us assume that there is an antigen (x) common to all human erythrocytes, and also that the specific antigenic substances or isoagglutinogens in the various groups are capable of acting as heteroagglutinogens and so cause the production of specific immune heteroagglutinins when injected into rabbits. Thus a rabbit injected with the cells of D. J., containing antigens x, b and c, might be expected to develop the corresponding agglutinins, X, B and C. Absorption of such an immune serum with the cells of D. J. should remove all of the agglutinin for those cells and for those of H. (xb) as well. On the contrary, absorption with the cells of H. should remove the agglutinin for those cells without, however, removing all of that for the cells of D. J. In a similar way, an immune serum produced by the injection of red cells from H. should be entirely deprived of agglutinin for both types of cells when absorbed with the cells of either H. or D. J. These theoretical considerations are presented in Table I.

The object of the experiments reported in this paper was: *To produce, by the immunization of rabbits, differential agglutinating sera for the two types of "Group II" cells as represented by the bloods of H. and D. J.*

TABLE I.

Theory of production of differential agglutinating sera for two types of "Group II" blood.

RABBIT			RESULTING IMMUNE SERUM						
Injected with		Serum develops agglutinins	Absorbed with R. B. C.		Agglutinin content after absorption	Agglutinates R. B. C.			
						Before absorption		After absorption	
R. B. C. of	Containing antigens		Gr. II (H.) containing antigens	Gr. II (D. J.) containing antigens		Gr. II (H.)	Gr. II (D. J.)	Gr. II (H.)	Gr. II (D. J.)
Group II (H.)	*xb*	*XB*	*xb*		None	+	+	0	0
				xbc	None	+	+	0	0
Group II (D. J.)	*xbc*	*XBC*	*xb*		0	+	+	0	+
				xbc	None	+	+	0	0

PROCEDURE

Eight adult rabbits were used in this work, all previously unused and apparently healthy, but only four of them developed an agglutinative titre sufficiently high to warrant the use of their serum in our tests. Just prior to the first injection, each rabbit was bled from an ear vein and the serum tested against the two types of "Group II" cells for the presence of normal heteroagglutinin. The animals then received intravenous injections of 1 c.c. of a 50 per cent suspension of thrice-washed red cells on the first, second and fourth days; on the eleventh day they were bled by aspiration from the heart; after inactivation for 30 minutes at 56°C., the sera were tested for agglutinative power against both types of "Group II" cells and also against cells from representatives of the other 3 accepted groups. Following these titrations a portion of each inactivated serum was absorbed at 37°C. with packed cells from H. and another portion with cells from D. J. In each instance thrice-washed cells were employed for this purpose and serum diluted 1 to 8. Small amounts of packed cells were added to the diluted serum and after a short time were removed by centrifugalization. This was repeated several times until microscopic tests showed that the supernatant fluid was no longer capable of agglutinating fresh cells of the type used for absorption. When the absorption was thus shown to be complete, a series of dilutions was made with the absorbed serum which was then titrated against each of the two types of "Group II" cells. Throughout this work all tests for the presence of agglutination were made in tubes containing serum, diluted or undiluted, and a 5 per cent suspension of red cells, in the proportion of 10 to 1 by volume. The tubes were placed in a water-bath at 37° C. for one hour; then, after a macroscopic reading, the tubes were shaken gently and a hanging-drop preparation made from each for microscopic examination. In the preliminary tests for the presence of normal agglutinin, the microscopic method gave positive results in somewhat higher dilutions than could be recognized by the macroscopic method, but after immunization of the rabbits and after absorption of the immune sera this difference was much less pronounced. We have recorded separately, therefore, the results of the macroscopic readings on the preliminary tests only.

RESULTS

The results of these experiments are shown in Table II; they may be recapitulated as follows:

Normal Agglutinin.—(a). Before the injections, the serum of each of the rabbits showed the presence of normal agglutinin for each of the two types of "Group II" cells.

TABLE II.

Experimental production of differential agglutinating sera for two types of "Group II" blood.

Rabbit number	R. B. C. used for injections	Before injections; agglutinative titre vs. R. B. C.		After injections; agglutinative titre vs. R. B. C.					After absorption with R. B. C.			
									Gr. II (H.)		Gr. II (D. J.)	
									Agglutinates R. B. C.		Agglutinates R. B. C.	
		Gr. II (H.)	Gr. II (D. J.)	Gr. II (H.)	Gr. II (D. J.)	Gr. I	Gr. III	Gr. IV	Gr. II (H.)	Gr. II (D. J.)	Gr. II (H.)	Gr. II (D. J.)
D 80	Group II (H.)	16-128	16-128	400	400	400	400	300	0	0	0	0
D 84	Group II (H.)	64-128	32-64	700	700	600	500	500	0	0	0	0
1	Group II (D. J.)	64-256	32-128	600	1700	400	130	130	0	304	0	0
D 83	Group II (D. J.)	64-128	64-256	1200	3000	500	120	100	0	480	0	0

The titre by macroscopic examination is shown in the first numbers in columns 3 and 4; the second numbers in these, and all numbers in succeeding columns, refer to results of microscopic readings.

(b). This normal agglutinin was recognizable in considerably higher dilution by the microscopic than by the macroscopic method.

(c). When tested by the macroscopic method, two of the rabbits (Nos. 1 and D84) showed a higher content of agglutinin for the cells of H. than for those of D. J., but the other two (Nos. D83 and D80) made no distinction between them.

(d). When tested by the microscopic method, two of the rabbits (Nos. 1 and D84) showed a higher content in normal agglutinin for the cells of H. than for those of D. J.; one (No. D80) showed the same titre for each type of cells and one (No. D83) gave a higher titre for the cells of D. J. than for those of H.

Immune Agglutinin.—(e). No definite proportion was found between the relative amount of normal agglutinin originally present for either type of "Group II" cells and the agglutinative titre obtained for these cells by immunization.

(f.) In the two rabbits (Nos. 1 and D83) injected with the cells of D. J., (1) the agglutinin for the cells of "Group III" and "Group IV" did not rise above the level of the normal agglutinin for "Group II" cells; (2) that for the cells of "Group I" which contain agglutinogen *b* did rise above that level; (3) that for both types of "Group II" cells rose markedly but was almost three times as strong for the particular type of cells used for injection as for the other type (8.5 : 3 and 10 : 4).

(g). In one of the rabbits (No. D80) injected with the cells of H., (1) the agglutinative power for the cells of "Group III" and "Group IV" rose above the level of the normal agglutinin for "Group II" cells; (2) that for "Group I" cells rose somewhat but no higher than that for "Group III" cells; (3) the same titre was obtained for both types of "Group II" cells but this was no higher than that for the cells of "Group I" and "Group III."

(h). In the other rabbit (No. D84) injected with the cells of H., (1) the agglutinative power for the cells of "Group III" and "Group IV" rose above the level of the normal agglutinin for "Group II" cells, but (2) did not reach that obtained for the cells of "Group I;" (3) the same titre was obtained for both types of "Group II" cells but this was higher than that for the cells of "Group I."

Absorption Experiments.—(i). After absorption with the cells of H., the two rabbits (Nos. 1 and D83) injected with the cells of D. J. showed no agglutinin for the cells of H. but still showed agglutinin for the cells of D. J.

(j). After absorption with the cells of D. J., the two rabbits (Nos. 1 and D83) injected with the cells of D. J. showed no agglutinin for either the cells of H. or those of D. J.

(k.) After absorption either with the cells of H. or with those of D. J., the two rabbits (Nos. D80 and D84) injected with the cells of H. showed no agglutinin for either type of "Group II" cells.

Comment

In essential detail these results are those which were anticipated as they are in entire agreement with the theoretical consideration presented above (compare Tables I and II). Despite this agreement, however, it is apparent that the experiments exhibit certain minor shortcomings, which may seem, perhaps, to detract from their value and render the results less striking. Among such deficiencies may be mentioned the following:

(a). Prior to immunization, the serum of each rabbit should have been (1) also tested for normal agglutinin against the cells of "Group I," "Group III" and "Group IV;" and (2) absorbed with, and then tested against the cells of "Group II" (H.), "Group II" (D. J.) and representatives of the other three accepted groups.

(b) After immunization, the serum of each rabbit should also have been absorbed with and then tested against cells from members of the other three accepted groups.

(c.) A higher agglutinative titre in the sera of the rabbits immunized against the cells of H. would have been desirable.

Thus, it was not conclusively shown that any of the rabbits actually developed immune heteroagglutinins which were specific for either type of "Group II" cells as distinguished from the cells of the other three accepted groups. It was demonstrated, however, that the rabbits injected with the cells of D. J. contained in their sera an agglutinin which was specific for the cells of D. J. (*A*—*bc*) as distinguished from those of H. (*A*—*b*), whereas the sera of rabbits injected with the cells of H. were incapable of effecting any such differentiation of the two types of "Group II" cells.

Likewise it was not proven that the apparently specific agglutination of the cells of D. J. was due to immune rather than normal heteroagglutinin, although this was strongly suggested by the fact that the agglutinative power of the rabbit sera was higher after absorption with the cells of H. than before immunization (Nos. 1 and D83). It is to be recalled, however, that *both* of the rabbits injected with red cells containing agglutinogen *c* later showed the presence of the corresponding agglutinin in their sera, and these rabbits *only*. To regard this as mere coincidence would be to put an unwarranted strain upon credulity.

The agglutinative power of the rabbits (Nos. 1 and D83) injected with the cells of D. J. was not so high as we had hoped it might be after absorption with the cells of H. It was, nevertheless, quite definite, and it should be recalled that the absorption was complete. In our experience, it is relatively easy to remove a large percentage of agglutinin by specific absorption, but the final traces present much more difficulty, necessitating the use of a disproportionately large amount of cells, particularly when the absorption is carried out by the repeated addi-

tion of small amounts of cells and at body temperature, as in this instance. A procedure of the sort just mentioned may also have another effect when applied to the separation of two agglutinins in the same serum; a diminution in the titre of the second agglutinin may be inadvertently but eventually produced if the specific absorption for the first agglutinin is carried too far or repeated too often. To what extent this is dependent upon the deterioration which inevitably occurs in diluted sera, particularly when not kept in the cold, we cannot say; that it may happen is a matter of observation. In these experiments, however, we desired to adhere rather closely to the technic of our borrowed method but felt that the absorption should be made complete for the particular type of cells used, believing that in work of this sort the contrast between *some* and *none* might be quite as striking as between *much* and *a little*.

An entire repetition of the experiments of Hooker and Anderson [2] was not contemplated but merely an application of their method of attack to a special problem of limited scope. Consequently, we are not qualified to report a complete confirmation of their work, but of its essential soundness both in theory and in practice we feel convinced as the result of our experiments.

In a former communication [1] attention was called to the result which might be anticipated when a patient is transfused with blood from a donor apparently belonging to the same group but whose cells contain an additional agglutinogen not present in those of the recipient. It was pointed out (1) that under these circumstances the recipient is likely to become immunized to the foreign agglutinogen and develop the corresponding agglutinin in his own blood; (2) that after this occurred an obvious incompatibility would be recognizable in matching the two bloods which would tend to discredit the results of previous tests; and (3) that if this precaution were ignored, an unfortunate result might be expected in any subsequent transfusion of that patient from the donor who had seemed entirely satisfactory at the primary transfusion. To those predictions the present work adds experimental evidence of the antigenic property of an agglutinogen (*c*) whose presence in the blood of D. J. would never be detected by ordinary matching. Confirmation is likewise afforded of our original observations concerning the agglutinogen content in the erythrocytes of these two types of "Group II" blood.

CONCLUSIONS

1. An agglutinin capable of differentiating the two types of "Group II" blood represented by the formulae *A—b* and *A—bc* has been demonstrated in the sera of rabbits after immunization.

2. This finding confirms our earlier impressions concerning the different agglutinogen content in these two types of "Group II" blood.

3. The bearing of these observations upon transfusion in human beings is discussed.

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XANTHOMA DIABETICORUM

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Xanthoma diabeticorum, according to Sutton,[1] "is exceedingly rare" while Stelwagon [2] refers to it "a rare eruption observed in diabetic individuals." Most of the standard textbooks on skin diseases mention it as an unusual disease.

The first description of this disease was given by Addison and Gull,[3] who observed a diabetic patient in 1848 with a skin eruption that "gave rise at the time to much discussion." Their description of the skin lesions is so accurate and clear that it still remains unsurpassed.

"At this time" (they wrote) "an eruption somewhat suddenly appeared on the arms, at first apparently of a lichenous character. In the course of ten days it had extended over the arms, legs and trunk, both anteriorly and posteriorly, also over the face and into the hair; it consisted of scattered tubercles of various sizes, some being as large as a small pea, together with shining colorless papules. They were most numerous on the outside and back of the fore-arm and especially about the elbows and knees, where they were confluent. Along the inner sides of the arms and thighs they were more sparingly present, and entirely absent from the flexures of the larger joints. Besides the compound character produced by the confluence of two or three large tubercles, many of the single ones had also a compound character, or appeared to have such, as shown by the prominent whitish nodules upon them. Some looked as if they were beginning to suppurate, and many were not unlike the ordinary Molluscum, but when incised with a lancet they were found to consist of firm tissue, which, on pressure, gave out no fluid save blood. They were of a yellowish color, mottled with a deepish rose-tint, and with small

capillary veins here and there ramifying over them. They were accompanied with a moderate degree of irritation, hence the apices of many were rubbed and inflamed."

Bristowe in 1866 [4] described a second case, and Gendre,[5] in 1880, reported another. Morris and Clarke,[6] in 1892, collected thirteen cases, and Johnston,[7] in 1895, reviewed twenty-one cases. Norman Walker [8] states that up to 1897 thirty cases had been reported. Among the many interesting contributions to this subject the articles of Johnston,[7] Robinson,[9] Schwenter-Trachsler,[10] Pusey and Johnstone,[11] Hamilton,[12] Lyon [13] and of Mook and Weiss [14] may be mentioned.

In reviewing the literature on xanthoma diabeticorum certain differences in opinion as to the correctness of the diagnosis in some cases is apparent. Schwenter-Trachsler (*l.c.*), who reviewed the literature in 1898, accepts only sixteen cases as authentic and lists six others as doubtful. Although his criteria for diagnosis may be too severe, yet certain cases have been described as xanthoma diabeticorum which probably have no claim to this title. Such cases as that of Hutchinson [15] described as "Xanthoma Diabeticorum without Glycosuria" and those of Robinson [16] and Sequeira [17] which showed no glycosuria should probably not be included. A survey of the literature with the exclusion of the very doubtful ones shows that up to the present seventy-four cases of xanthoma diabeticorum have been described.

The earlier students of this disease concerned themselves largely with the clinical picture. Abraham [18] pointed out its rarity among women, although I have found at least ten examples in women in the literature up to date. Most observers describe its disappearance with improvement in the diabetes, although interesting exceptions are noted. Abraham [19] describes a patient in whom the diabetic symptoms did not appear until one year after the eruption. Pisko,[20] describing the same case reported three years earlier by Sherwell and Johnston,[21] noted that the tumors were stationary, although the patient remained sugar-free. Lancashire [22] describes a case in which there was an increase in the number of nodules with an improvement in the patient's condition. Renon and Follet [23] describes the development of xanthomata in a patient who had been sugar-free for fifteen months, while Töpfer [24] reports a case in which an increase in the glycosuria was accompanied by a disappearance of the xanthomata.

In addition to describing the clinical picture of this disease a number of observers have also studied the microscopic appearance of the lesions. Bristowe,[4] who reported the second case of xanthoma diabeticorum, describes the lesions as showing microscopically a "dense fibrillated texture, studded more or less with oil globules of various sizes." Morris [25] gives a good description of the microscopic sections from his case in which inflammatory signs were prominent microscopically, while Tholen [29] stated that the sections showed no inflammation and expressed the view that the xanthoma was a product of the lipoidemia. Hamilton [12] reported that in his case the sections showed only fibrosis and no cholesterin. No mention, however, is made of any study of the sections by special staining methods.

Observations upon the appearance of the blood or upon the blood chemistry of patients with xanthoma diabeticorum have been few in number. Duckworth [30] describes lipemia as a postmortem finding in one case; Lyon [13] and Mook and Weiss [14] noted lipemia in their patients. I have found but three records of the blood cholesterol in xanthoma diabeticorum; Griffith [31] describing a patient with a blood cholesterol of 397 milligrams, per 100 c.c., Mook and Weiss[14] noting a blood cholesterol of 780 milligrams per 100 c.c. in a patient, and Nicholson [33] reporting a blood cholesterol of 1260 milligrams per 100 c.c.

I have recently had an opportunity of studying three cases of xanthoma diabeticorum. These are of especial interest because of studies made upon the blood cholesterol and also because two of the patients were treated with insulin. Sections of the xanthoma nodules were made in two instances and studied carefully with a variety of stains.

The first patient was a man, aged forty-three, a Jew, admitted to the Bell Memorial Hospital on March 19, 1923, in diabetic coma and obviously *in extremis*. The history obtained was not complete, but it was learned that he had had diabetes for two years and had had a skin eruption for two months. This skin eruption appeared rather suddenly over the arms and spread in a few days to the chest, back, thighs and legs. The patient's family thought at first they were small boils, but observed that they "neither broke nor disappeared."

On examination this patient presented a very profuse eruption of small hard discrete nodules, orange yellow in color and scattered over both arms, the chest, thighs and legs. They were most numerous over the flexor surfaces of the legs and arms. This patient's blood serum was milky white; it presented a marked gross lipemia, and the blood cholesterol (blood plasma) was 432 milligrams per 100 c.c. This man died three hours after admission to the Hospital.

The second patient was a man, aged twenty-five, who was admitted to the Bell Memorial Hospital on April 11, 1923, for treatment. He had had diabetes for two years and six months before admission, and had noted the presence of small yellowish nodules over both forearms. He was very drowsy when first seen, his respirations were 20 per minute and he presented the clinical picture of a beginning diabetic coma.

Both forearms showed numerous small, hard, discrete, yellowish nodules especially numerous over the flexor surfaces. The patient's blood sugar on admission was

293 milligrams per 100 c.c., the carbon-dioxide tension was 12 volumes per cent (Van Slyke) and the blood plasma cholesterol was 632 milligrams per 100 c.c. There was a marked lipemia and the blood, when taken from the finger-tip for a blood count, was of a yellowish gray color suggesting the "pus-like" blood of a patient suffering from a severe leukemia.

This patient was immediately started upon insulin (Lilly) and the result is shown in Fig. 1. The insulin therapy was followed by a disappearance of the lipemia in four days, a prompt fall in the blood cholesterol to a normal value, disappearance of the hyperglycemia and glycosuria and a return of the low carbon-dioxide tension to normal. He was discharged from the hospital on May 9, 1923, in excellent condition, the skin lesions having entirely cleared up.

A section from one of the excised xanthoma nodules showed marked evidences of inflammation. The corium was thickened; extensive keratinization had taken place; there were areas of small-round-celled infiltration; marked fibrous changes were present and the characteristic large clear xanthoma cells were recognized with difficulty. In general appearance the sections resembled closely those of the third case, although the inflammatory changes were more marked.

The third patient was a man, aged twenty-six, who was admitted to the Bell Memorial Hospital on March 23, 1923. He had suffered from diabetes for three years and had first noted a skin eruption on his arms in July, 1922. This eruption presented itself in the form of small discrete firm yellowish nodules 2 to 5 millimeters in diameter and larger elevated areas, 1 to 4 centimeters in diameter, which had been produced by coalescence of the smaller nodules. These larger areas were especially numerous, over the elbow, (Fig. 2) the knees and the buttocks. The palms of both hands were studded with smaller yellowish orange nodules (Fig. 3), many of them with a whitish, somewhat translucent apex. The lines of the palms and of the wrists were accentuated by a deposit of orange-colored pigment just under the skin. Some of the larger areas were also present on the hands and here, as over the elbows and knees, they showed the "small capillary veins here and there ramifying over them" as described by Addison and Gull.[3]

Several of the larger nodules were excised for microscopic study and a typical section is shown in Fig. 4.

This section, stained with hematoxylin and eosin, shows a marked fibrosis with infiltration of small mononuclear leukocytes, the xanthoma cells being inconspicuous. A section stained with Sudan III (Fig. 5) shows a very large number of deep-red-staining cells and many smaller cells staining a lighter red or orange. There is a great variation in the sizes of the cells taking this stain and also marked variations in the staining intensity. Sections stained with Nile Blue Sulphate show that some areas stain a deep blue, while others stain an intense red and various shades of red. Cells are also seen containing crystals which are doubly refractive and the tissue, when stained by the method of Golodetz, gives a positive reaction for cholesterol.

A study of the sections of tissue by these special methods indicates that these nodules are composed of neutral fat, fatty acids and lipoid bodies, and contain cholesterol.

This patient was placed upon insulin therapy and the results of treatment are shown in Fig. 6. He also responded with a prompt fall in blood sugar from 333 milligrams per 100 c.c. to normal, and the blood plasma cholesterol from 533 milligrams to 150 milligrams per 100 c.c. to normal. Many of the smaller nodules disappeared, but no change was noted in the larger confluent areas. This result is in accord with the experience of Balzer and Marie,[32] who describe a case in which the skin lesions consisted of "éléments miliaires" and of "éléments lenticulaires," the former clearing up rapidly when the patient became aglycosuric, the latter proving much more resistant to treatment.

With the consent of our patient three of the large areas on the left elbow were injected with a small quantity of ether. The nodules softened almost immediately and the following day were covered by a crust. This treatment was followed by some soreness, the skin about the treated nodules became swollen and, because of the discomfort, this method of therapy was not repeated. A striking immediate result was the greatly increased vascularity of the skin around the treated nodules.

Shortly afterwards, on April 27, 1923, the patient was discharged from the hospital and continued the insulin therapy at his home, when his blood sugar was 167 milligrams per 100 c.c. and his blood plasma cholesterol 117 milligrams per 100 c.c. He was last seen on July 10, 1923. At this time the process on the right elbow and on the hands had undergone but little change, the lesions on the left elbow, however, had for the most part disappeared, the results, we believe, of the increased vascularity of this area following the injection of ether.

All of these observations indicate that the clearing up of xanthoma diabeticorum is dependent upon two factors, a disappearance of lipemia and hypercholesterinemia, and the presence of a good blood supply to the skin lesions. In our first patient the disappearance of the small xanthoma nodules that had a relatively good blood supply followed promptly the disappearance of the hypercholesterinemia. In the second patient, insulin produced a prompt fall in the blood cholesterol; the smaller nodules disappeared but the larger areas having a very poor blood supply were not absorbed.

This study of the close relationship between lipemia, the blood cholesterol and xanthoma diabeticorum, explains to a certain extent the seemingly conflicting re-

sults which have followed the disappearance of glycosuria. Xanthoma diabeticorum is related more closely to the disturbed fat metabolism of diabetes mellitus than to the disturbances of carbohydrate metabolism. It is highly probable that some patients, even after they become aglycosuric, continue to have a hypercholesterinemia of sufficient degree to produce xanthomata.

The question at one time hotly debated, as to whether xanthoma diabeticorum is the result of an inflammatory reaction produced by a slight trauma or whether it is solely due to the lipoidemia, needs little discussion. Both factors are commonly present, and without lipoidemia xanthoma diabeticorum probably does not occur.

The effect of trauma upon a patient with lipoidemia was shown recently in a striking fashion. A boy of sixteen, suffering from a severe diabetes with a blood sugar of 322 milligrams and a blood cholesterol of 400 milligrams per 100 c.c., was bitten in two places on the forearm by a mosquito. Two small inflammatory nodules were produced and a few days later a deposit of yellowish orange pigment appeared, the nodules became quite hard and presented the typical appearance of the lesions of xanthoma diabeticorum. This boy's skin, particularly of the face, the palms of the hands and the soles of the feet, showed a yellowish pigmentation (xanthosis diabetica). The nodules cleared up promptly under insulin therapy.

The blood sugar determinations referred to in this paper were made according to the method of Folin and Wu; the carbon dioxide tension in the blood by the method of Van Slyke, and the cholesterol by the method of Myers and Waddell.

Conclusions

Seventy-four cases of xanthoma diabeticorum have been collected from the literature.

Three cases of xanthoma diabeticorum studied in this report showed lipemia and hypercholesterinemia.

Insulin clears up lipemia and hypercholesterinemia.

The clearing up of xanthoma diabeticorum depends partly upon a disappearance of the lipemia and lipoidemia, and partly upon the degree of blood supply which permits absorption of the substances in the xanthomatous lesions.

My thanks are due to Dr. C. C. Dennie, who kindly referred one of these patients, and to Dr. H. R. Wahl for much assistance and valuable suggestions in the study of these sections.

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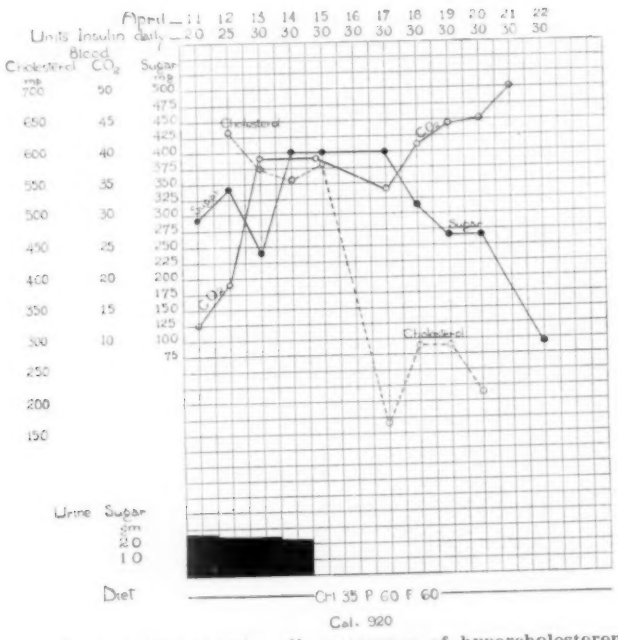

Fig. 1.—Chart showing disappearance of hypercholesterenemia, hyperglycemia and glycosuria with rise in carbon-dioxide tension following insulin therapy.

Fig. 2.—Photograph of skin lesions over elbows of patient No. 3.

Fig. 3.—Drawing of lesions on hand of patient No. 3.

Fig. 4.—Microphotograph of section of xanthoma nodule. Note large clear cells, also evidences of inflammation. (Spencer oc. 10 x, obj. 4 mm.). Stain Hematoxylin and Eosin.

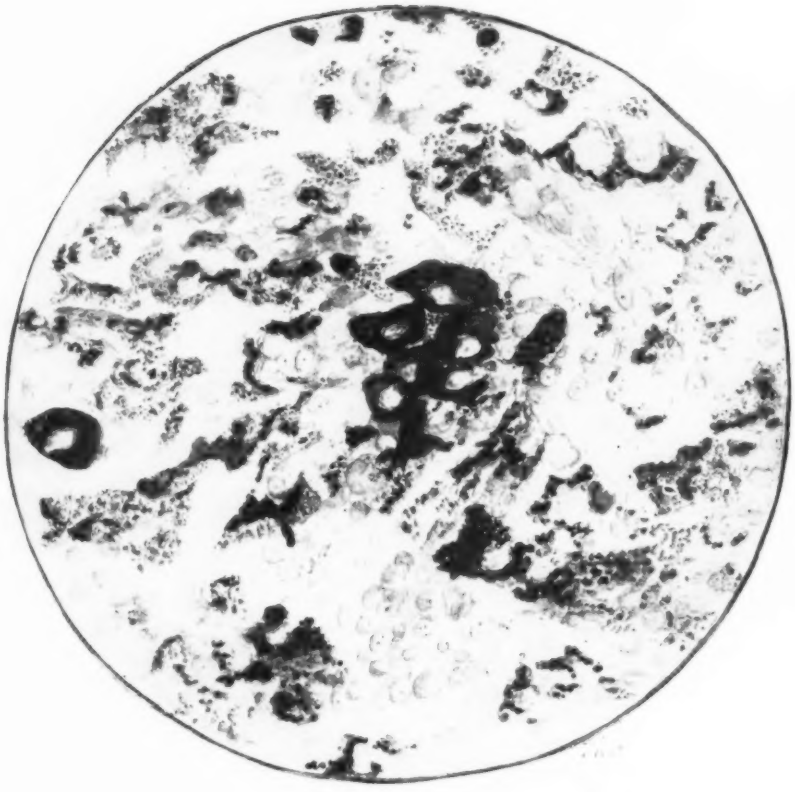

Fig. 5.—Drawing of section of xanthoma nodule stained with Sudan III. (Spencer oc. 10 x. obj. 4 mm.)

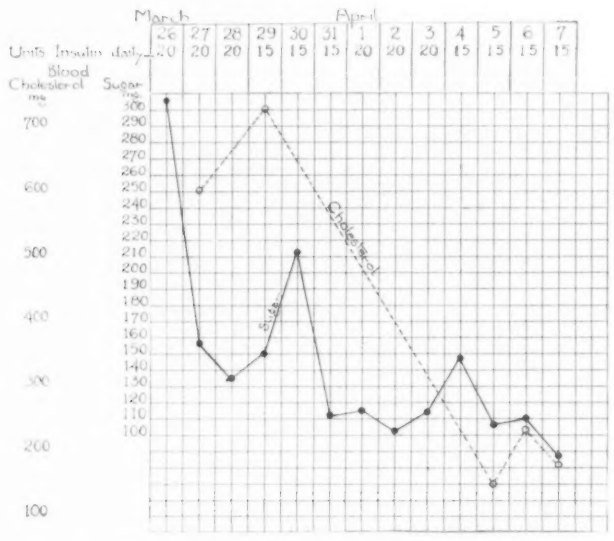

Fig. 6.—Chart showing disappearance of hypercholesterenemia and hyperglycemia following insulin therapy.

LIST OF CASES OF XANTHOMA DIABETICORUM

Case No.	Author	Year	Reference
1	Addison and Gull	1850	Guy's Hosp. Rep., 1850, VII, 265.
2	Bristowe	1866	Tr. Path. Soc. Lond., 1866, XVII, 414.
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4	Hillairet	1882	Chambard: Ann. de dermat. et syph., 1884, V, 348.
5	Morris	1883	Tr. Path. Soc. Lond., 1883, XXXIV, 278.
6	Hardaway	1884	St. Louis Cour. Med., 1884, XII, 289.
7	Aubert	1884	Chambard: Ann. de dermat. et syph., 1884, V, 348.
8	Barlow	1888	Brit. J. Dermat., 1888, I, 3.
9	Besnier	1889	Ann. de dermat. et syph., 1889, 2 s., X, 435.
10	Robinson	1891	Brit. J. Dermat., 1891, III, 106.
11	Morris and Clark	1891	Brit. M. J., 1891, II, 1310.
12	Crocker	1892	Brit. J. Dermat., 1892, IV, 253.
13	Payne	1893	Brit. J. Dermat., 1893, V, 325.
14	Tims	1893	Brit. J. Dermat., 1893, V, 325.
15	Pollitzer	1893	Brit. J. Dermat., 1893, V, 368.
16	Hallopeau	1893	Ann. de dermat. et syph., 1893, IV, 935.
17	Jamieson	1894	Brit. J. Dermat., 1894, VI, 289.
18	Johnston	1895	J. Cutan. & Genito-Urin. Dis., 1895, XIII, 401.
19	Schamberg	1895	J. Cutan. & Genito-Urin. Dis., 1895, XIII.
20	Gans	1896	Therap. Monatsh., 1896, V, 15.
21	Darier	1896	Iconog. d. mal. cutan. et syph., 1896, 53.
22	Walker	1897	Brit. J. Dermat., 1897, IX, 461.
23	Colombini	1897	(quoted by Schwenter-Trachsler).
24	Schwenter-Trachsler	1898	Monatsh. f. Prakt. Dermat., 1898, XXVII, 209.
25	Niermann	1898	I. D., Würzburg, 1898.
26	Quivy	1898	Thèse de Paris, 1898.
27	Schwenter	1898	Deutsche Med. Wchnschr., 1898, XXIV, 248.
28	Fabry	1899	Verhanal. d. Gesellsch. deutsch. Naturf., 1899, LXX, 323
29	Hallopeau	1899	Bull. Soc. franç. de dermat. et syph., 1899, X, 289.
30	Krzysztalswicz	1899	Monatsh. f. prakt. Dermat., 1899, XXIX, 201.
31	Bosellini	1900	Clin. Med. Ital., 1900, XXXIX, 673.
32	Bosellini	1900	Clin. Med. Ital., 1900, XXXIX, 673.
33	Bosellini	1900	Clin. Med. Ital., 1900, XXXIX, 673.
34	Herman	1900	Memphis Lancet, 1900, IV, 74.
35	Sherwell and Johnston	1900	J. Cutan. & Genito-Urin. Dis., 1900, XVIII, 387.
36	Abraham	1901	Tr. Dermat. Soc. Gr. Brit., 1901, VIII, 18.
37	Marullo	1903	Dermat. Ztschr., 1903, X, 354.
38	Fox	1903	J. Cutan. Dis., 1903, XXI, 233.
39	Abraham	1903	Brit. J. Dermat., 1903, XV, 456.
40	Leven	1903	Arch. f. Dermat. u. Syph., 1903, LXVI, 61.
41	Derlin	1904	München. med. Wchnschr., 1904, LI[2], 1636.
42	Gaucher et Lacapere	1904	Bull. Soc. franç. de dermat. et syph., 1904, XV, 185.
43	Duckworth	1905	Tr. Clin. Soc., 1905, XXXVIII, 183.
44	Adamson	1905	Westminst. Hosp. Rep., 1905, XIV, 84.
45	Spillmann	1905	Mém. d. soc. méd. d. Nancy, 1905, p. 108.
46	Proctor	1905	Lancet, 1905, II, 1392.
47	Hallopeau	1906	Bull. Soc. franç. de dermat. et syph., 1906, XVII, 283.
48	Abraham	1906	Postgraduate, 1906, XXI, 583.
49	Lancashire	1907	Brit. J. Dermat., 1907, XIX, 269.
50	Zak	1907	Russk. J. Kozhn, etc., 1907, XIII, 365.
51	Fox	1907	Tr. Internat. Dermat. Cong., 1907, I, 448.
52	Shepherd	1907	Tr. Internat. Dermat. Cong., 1907, I, 449.
53	Galloway	1908	Proc. Roy. Soc. Med., 1908, II, Dermat. Sect. 139.
54	Pusey and Johnstone	1908	J. Cutan. Dis., 1908, XXVIII, 552.
55	Crocker	1909	Proc. Roy. Soc. Med., 1909, Dermat. Sect., p. 85.
56	Balzer and Marie	1910	Bull. Soc. franç. de dermat. et syph., 1910, XXI, 4.
57	Wirsing	1911	Berl. klin. Wchnschr., 1911, LVIII, 1398.
58	Tholen	1911	L. D., Leipzig, 1912.
59	Fordyce	1914	J. Cutan. Dis., 1914, XXXII, 804.
60	de Buy Wenniger	1914	Nederl. Tydschr., v. Geneesk., 1914, II, 1812.
61	Fahr	1919	Deutsche med. Wchnschr., 1919, XLV, 1373.
62	Sibley	1920	Proc. Roy. Soc. Med., 1920, XIII, Sect. Dermat., 83.
63	Shamberg	1920	Arch. Dermat. & Syph., 1920, I, 98.
64	Oemsby and Finnerud	1921	Arch. Dermat. & Syph., 1921, III, 692.

Case No.	Author	Year	Reference
65	Harrold	1920	M. J. Australia, 1920, I, 84.
66	Griffith	1922	Arch. Pediat., 1922, XXXIX, 297.
67	Hamilton	1922	M. J. Australia, 1922, I, 385.
68	Lyon	1922	Edinburgh M. J., 1922, N. S., XXVIII, 168.
69	Moore	1922	Brit. M. J., 1922, II, 1176.
70	Mook and Weiss	1923	Arch. Dermat. & Syph., 1923, VIII, 19.
71	Mook and Weiss	1923	Arch. Dermat. & Syph., 1923, VIII, 19.
72	Mook and Weiss	1923	Arch. Dermat. & Syph., 1923, VIII, 19.
73	Nicholson	1923	Clifton M. Bull., 1923, IX, 12.
74	Milne	1923	Reported at Kansas Medical Society, May 2, 1923.

NOTE: The case of Hutchinson (*l.c.*), Robinson (*l.c.*), Sequeira (*l.c.*), Fox (Lancet 1879, II, 688) and Cavay (Brit. J. Dermat., 1888, I, 76), commonly referred to in the literature, have not been included, because the patients showed no glycosuria nor any evidence of diabetes. An article by Ineda and Kikuti (Iji Shinbun, 1903, 569) was not accessible and their patients are not included in this table.

NOTES ON NEW BOOKS

Respiration. By J. S. Haldane. *(New Haven, Yale University Press, 1922).*

In this volume Dr. Haldane has presented his Silliman Lectures at Yale (1915) amplified and revised in content so as to include researches up to date of publication. It is a monograph on respiration; and as such deals mainly with an account of Dr. Haldane's life work in physiology, for a great part of the enormous advance in the special physiology of respiration is due to the author and his pupils.

This volume begins with an interesting historical account of the development of our knowledge of the function of respiration and the respiratory mechanism. The physico-chemical aspects of respiration are treated in chapters on carbon dioxide and the regulation of breathing; on the blood as a carrier of oxygen and of carbon dioxide; and on blood reaction and breathing; while the physiological and medical aspects (if such a division is permissible) are treated in chapters on the effects of want of oxygen; on the causes of anoxaemia; on the disputed topic of gas secretion in the lungs which most investigators have discarded but which Haldane has held tenaciously, though forced to modify it somewhat in form; and on blood circulation and breathing. The last portion of the book may be classed as applied physiology, and contains chapters on air of abnormal composition; effects of high atmospheric pressures; effects of low atmospheric pressures as well as an appendix of analytical methods. The book closes with a chapter outlining Haldane's philosophy of physiology and is entitled "General Conclusions."

Aside from the instructions afforded by the book on the theory of respiration, the reader will be impressed by several things. First, Haldane has followed the precepts of Voit and has arranged his experiments so as to use the unanesthetized normal mammalian organism and has used active normal men (himself and his students) as his subjects. Secondly, the author has taken up Claude Bernard's concept of the capacity of the organism for the "preservation of the conditions of life in the internal environment" to differentiate himself both from vitalists and mechanists. Thirdly, one cannot help but be impressed with the extent to which interest in industrial problems, such as the effects of mine air, of air in tunnels, or of sudden compression or decompression of air has led Haldane to very fine purely scientific and theoretical work.

The book will be read with pleasure by those interested in the subject of respiration or its clinical applications.

B. S. N.

Cunningham's Text-Book of Anatomy. Fifth Edition. Revised. *(New York, William Wood and Company, 1923).*

All the chapters in the fifth edition of this book have been revised and the ones on Osteology, Myology, the Peripheral Nervous System and the Ductless Glands rewritten. Those on the Peripheral Nervous System by S. E. Whitnall, and the Ductless Glands by J. T. Wilson, are much improved.

Professor Whitnall has incorporated in his chapter much of the newer knowledge concerning the classification of cranial nerves. One is pleased to note the mention of the vomero-nasal and terminal nerves, although the incorporation of the latter under Nervi olfactorii seems unjustifiable. This section of the book has been enhanced by the addition of several new illustrations, notably one showing the formation of the brachial plexus from which a conception of the primary trunks is readily gained.

In the chapter on Ductless Glands proper emphasis is laid on the adrenal (chromaffin) system and the interrenal (cortical) system, portions of which unite in higher vertebrates to form the suprarenal glands. The hypophysis, the pineal body, and the pharyngeal group of ductless glands are adequately treated. Inadvisable, we believe, is the establishment of a lymphoid group of ductless glands under which would fall of necessity the spleen, thymus, tonsils, lymph-glands, hemolymph nodes and the scattered lymphoid nodules of the body. There is only the scantest evidence for recognizing the thymus as a gland of internal secretion, much less any of the others. The spleen, so far as we know, is concerned only in hemopoiesis and should be discussed in the chapter dealing with that subject. Even so eminent an authority as Arthur Biedl does not include the spleen in his encyclopedic work on internal secretion, and the B. N. A. terminology has done wisely in separating the "glandulae vasculares"—the tissues above enumerated—from the organs possessing an undoubted function of internal secretion.

The section on Myology which has been slightly revised is excellent. The discussions of muscle mechanics deserve commendation. A plate showing the attachments of the muscles of the skull is a helpful addition.

On the whole this book can be recommended to the student and teacher of anatomy. It is as complete and well-organized a work of this kind as exists in the English language. The illustrations are numerous, clear and well reproduced.

G. B. W.